

# *In vitro* Antioxidant Potential of *Abrus precatorius* L. and *Asystasia gangetica* (L.) T. Anderson

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**Abstract:** Antioxidant substances are an important part of human life as it plays a key role in nutraceuticals and also help to defend free radicals present in our body. The present study highlights the need to find potent natural antioxidants from medicinal plants. Different extracts of *Abrus precatorius* L. (Fabaceae) and *Asystasia gangetica* (L.) T. Anderson (Acanthaceae) were successively prepared using petroleum ether, benzene, chloroform, and ethanol. It was evaluated for antioxidant activities using various assays. Maximum extractable total phenolics and flavonoids were recorded in varied extracts of *A. precatorius* and *A. gangetica*. The extracts also showed efficient phosphomolybdenum reduction, reducing power activity, nitric oxide, and hydrogen peroxide radical scavenging properties. It is very clear from the results that the studied plants *A. precatorius* and *A. gangetica* have remarkable medicinal uses with extraordinary potential for pharmaceuticals. Further detailed studies will pave the way to promote natural drugs for health benefits.

**Keywords:** antioxidant; nutraceuticals; free radicals; pharmaceuticals.

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## 1. Introduction

Free radical is capable of independent existence because it contains unpaired electrons. This reacts with another molecule by taking or giving electrons and is involved in various pathological conditions [1]. Free radicals contribute to aging and degenerative diseases such as cancer, immune system decline, brain dysfunction, and cardiovascular disease [2, 3]. Antioxidants provide various useful compounds that control free radicals. Medicinal plants produce different secondary metabolites with antioxidant properties. Natural antioxidants in plant extracts protect the cells from oxidative damage and scavenge harmful free radicals in our bodies. The antioxidant activities of plants are linked with phenolic compounds, flavonoids, anthocyanins, and tannins. Numerous pieces of evidence by a large number of screening tests confirmed the *in vitro* antioxidant activities [4, 5]. Epidemiological studies also revealed that antioxidant compounds possess various properties, including antitumor, analgesic, anti-inflammatory, antimutagenic, anti-atherosclerotic, and antimicrobial [6, 7].

*Abrus precatorius* L. (Fabaceae), commonly known as Indian licorice, is a climbing shrub native to Indonesia. It grows in tropical and subtropical areas of the world and is distributed throughout India. Roots possess the diuretic activity and are also used in preparations prescribed for jaundice, haemoglobinuria, and gonorrhoea [8]. Glycyrrhizin, the principal constituent of *A. precatorius* is used as a substitute for *Glycyrrhiza glabra* in catarrhal infections and cough [9]. Lectins isolated from *A. precatorius* contain immunostimulant properties [10]. The seeds of *A. precatorius* are used to treat arthritis, eye diseases, diabetes, jaundice, leucoderma, and poisonous bite in Siddha medicine [11].

*Asystasia gangetica* (L.) T. Anderson (Ganges Primrose) is a fast-growing herbaceous plant belonging to the family Acanthaceae. It is cosmopolitan in occurrence. The leaves contain huge amounts of proteins, minerals, lipids, and fibers. The plant is used ethnomedicinally to treat rheumatism, heart pain, vermifuge, and stomach pains [12]. Pharmacological studies confirmed that the leaves of *A. gangetica* contain anti-inflammatory and bronchospasmolytic properties. The leaves extract also inhibit histamine and serotonin-induced contractions of the guinea pig trachea [13]. With this background, the present study focuses on evaluating the antioxidant potential of *A. precatorius* and *A. gangetica* and their usage in various traditional systems of medicine.

## 2. Materials and Methods

### 2.1. Collection of plant materials.

Healthy plant specimens of *Abrus precatorius* L. (Fabaceae) and *Asystasia gangetica* (L.) T. Anderson (Acanthaceae) were collected from Marunthuvazhmalai, Kanyakumari District, Tamil Nadu, India. They were washed well initially with tap water to remove the adhered debris and then with distilled water. The plant samples were dried using blotting paper and kept at room temperature under shade for 15 days. The shade dried plant samples were ground to a fine powder using a mechanical grinder.

### 2.2. Preparation of extracts.

30 g of airdried powder was extracted successively with 180 mL of solvents viz., petroleum ether, benzene, chloroform, and ethanol using soxhlet apparatus for 8 h at a temperature not exceeding the boiling point of the solvent. The extracts were frozen and freeze-dried.

### 2.3. Determination of total phenolics.

The total phenolic content present in *A. precatorius* and *A. gangetica* was determined according to the method described by Janakiraman and Johnson [14]. 100-200  $\mu$ L aliquots were taken in test tubes and made up to 1 mL with distilled water. 0.5 mL of Folin-Ciocalteu reagent and 2.5 mL of sodium carbonate solution (20%) were added to each tube. The reaction mixture was vortexed, and the test tubes were placed in the dark for 40 min. The absorbance was noted at 725 nm against blank. The analysis was performed in triplicates, and the results were expressed as Gallic Acid Equivalents (GAE).

#### 2.4. Estimation of total flavonoids.

The flavonoid contents of various extracts of *A. precatorius* and *A. gangetica* were determined by the method described by Johnson et al. (2020) [15]. 500 µL of extracts were taken in different test tubes. 2 mL of distilled water and 150 µL of 5% NaNO<sub>2</sub> were added to all the test tubes. The tubes were incubated at room temperature for 6 min. After incubation, 150 µL of AlCl<sub>3</sub> (10%) was added to all the test tubes, including blank. All the tubes were again incubated for 6 min at room temperature. Finally, 2 mL of 8% NaOH was added, and it was made up to 5 mL using distilled water. The contents were vortexed well and allowed to stand for 15 min at room temperature. The absorbance was recorded at 510 nm using a spectrophotometer. The total flavonoid contents were expressed in GAE.

#### 2.5. Phosphomolybdenum assay.

The antioxidant potential of *A. precatorius* and *A. gangetica* was evaluated using the green phosphomolybdenum complex formation [16]. Aliquots of 100 µL extracts (in 1 mM DMSO) were combined with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in a 4 mL vial. The vials were sealed and incubated in a water bath at 95°C for 90 min. The samples were cooled to room temperature. The absorbance was noted at 695 nm against a blank. The results are expressed as g of ascorbic acid (AA) equivalents / 100 g extract.

#### 2.6. Nitric oxide radical scavenging activity.

The nitric oxide scavenging activity of various extracts of *A. precatorius* and *A. gangetica* on nitric oxide radical was measured using the standard method [17]. In phosphate-buffered saline, sodium nitroprusside (10 mM) was mixed with different concentrations (50-250 µL) of plant extracts. It was incubated at room temperature for 150 min. Griess reagent (0.5 mL), containing 1% sulphanilamide, 2% H<sub>3</sub>PO<sub>4</sub>, and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride was added to the mixture after incubation time. The absorbance of the formed chromophore was measured at 546 nm. BHT and rutin were used as a positive and negative control. Radical scavenging activity was expressed as the inhibition percentage of free radical by the extracts. The percentage inhibition activity was calculated using the formula:

$$\% \text{ radical scavenging activity} = [(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] \times 100$$

#### 2.7. Scavenging of hydrogen peroxide.

The ability of *A. precatorius* and *A. gangetica* extracts to scavenge hydrogen peroxide was determined according to the standard protocol [18]. 100-200 mg/mL extracts were added to hydrogen peroxide solution (0.6 mL) prepared in phosphate buffer (pH 7.4). After 10 minutes, the absorbance was recorded at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage inhibition activity was calculated using the formula:

$$\% \text{ scavenging activity} = [(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] \times 100$$

### 2.8. Reducing power.

The reducing power of different extracts *A. precatorius* and *A. gangetica* was determined according to the standard method reported by Bhalodia *et al.* (2013)[19]. 20-100 µL of extracts were taken in 1 mL of phosphate buffer, and 5 mL of 0.2 M phosphate buffer (pH 6.6) was added. To this, 5 mL of 1% potassium ferricyanide solution was added. The mixture was incubated at 50°C for 20 min. After an appropriate incubation period, 5 mL of 10% trichloroacetic acid was added. The contents were centrifuged at 1000 rpm for 10 min. The upper layer of the supernatant (5 mL) was mixed with 5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance of the reaction mixture was noted using a spectrophotometer at 700 nm.

### 2.9. Statistical analysis.

The results obtained from the different antioxidant assays were presented as triplicate determinations ± standard deviation (SD).

## 3. Results and Discussion

The total phenolic contents present in different extracts of *A. precatorius* and *A. gangetica* were presented in Table 1. In *A. precatorius*, the maximum extractable total phenolics (419.04 mg GAE/g) were recorded in ethanolic extracts followed by chloroform and petroleum ether extracts. Benzene extracts gave the lowest level of total phenolics (58.73 mg GAE/g). In *A. gangetica*, the highest amount of total phenolic contents was observed in benzene extracts (328.57 mg GAE/g), whereas petroleum ether and ethanolic extracts demonstrated a moderate level of phenolics. Chloroform extracts showed the minimum amount of phenolics (60.31 mg GAE/g). Phenolic compounds are one of the richest groups of secondary metabolites present in the plant kingdom [20]. It possesses antioxidant and free radical scavenging abilities, which potentially have numerous beneficial implications for human health [21]. Phenolic compounds act as natural antioxidants by helping to neutralize free radicals present in our body, and it was confirmed through various epidemiological studies. The multiple hydroxyl groups and their arrangement around the phenolic molecule help in scavenging free radicals [22, 23]. In the present study, the quantitative analysis determined the presence of phenolic compounds in all the four tested extracts of *A. precatorius* and *A. gangetica*. It suggests that the selected two plants are a promising source of effective antioxidants.

**Table 1.** Total Phenolics and Flavonoids present in *A. precatorius* and *A. gangetica*.

Extracts	Phenolics (mg GAE/g ± SD)		Flavonoids (mg GAE/g ± SD)	
	<i>A. precatorius</i>	<i>A. gangetica</i>	<i>A. precatorius</i>	<i>A. gangetica</i>
Petroleum ether	326.19 ± 9.91	217.46 ± 5.49	453.33 ± 6.66	160.00 ± 17.63
Benzene	58.73 ± 7.27	328.57 ± 12.59	86.66 ± 13.33	104.44 ± 13.87
Chloroform	361.90 ± 12.59	60.31 ± 7.27	342.22 ± 16.77	302.22 ± 3.84
Ethanol	419.04 ± 4.76	139.68 ± 7.27	153.33 ± 6.66	237.77 ± 10.18

**Table 2.** Phosphomolybdenum assay of *A. precatorius* and *A. gangetica*.

Extracts	<i>A. precatorius</i>	<i>A. gangetica</i>
Petroleum Ether	60.3 ± 2.3	4.1 ± 0.6
Benzene	20.2 ± 1.1	21.0 ± 1.3
Chloroform	25.5 ± 1.7	13.1 ± 1.7
Ethanol	65.9 ± 0.6	53.9 ± 1.1

**Table 3.** NO radical scavenging activity of *A. precatorius* and *A. gangetica*.

Extracts	<i>A. precatorius</i>	<i>A. gangetica</i>
Petroleum Ether	53.4 ± 1.46	55.3 ± 2.23
Benzene	87.9 ± 0.84	75.2 ± 1.46
Chloroform	82.0 ± 2.23	81.5 ± 3.03
Ethanol	67.4 ± 1.68	92.2 ± 0.84

**Table 4.** H<sub>2</sub>O<sub>2</sub> scavenging activity of *A. precatorius* and *A. gangetica*.

Extracts	<i>A. precatorius</i>	<i>A. gangetica</i>
Petroleum Ether	94.8 ± 0.4	96.3 ± 0.4
Benzene	75.8 ± 1.3	81.7 ± 0.7
Chloroform	90.8 ± 0.6	88.3 ± 0.6
Ethanol	96.8 ± 0.2	61.0 ± 0.2

Total flavonoid contents of various extracts of *A. precatorius* varied considerably from 86.66 to 453.33 mg GAE/g extract. The highest amount of flavonoids (453.33 mg GAE/g) were present in petroleum ether extracts, followed by chloroform and ethanolic extracts. Among the different extracts analyzed in *A. gangetica*, the flavonoids content was found to be maximum (302.22 mg GAE/g) in chloroform extracts, whereas the other extracts showed a moderate level of flavonoids (Table 1). Flavonoids are the broadly distributed group of plant phenolic compounds, and it acts as an effective antioxidant [24]. Flavonoids are responsible for protecting plants against various stresses. It helps in the interaction between the plant and its environment and plays an important role in exhibiting a variety of biological functions [25]. Flavonoids are not essential for the survival of the plant. They are biologically active and assist in the transport of auxin [26]. Flavonoids are responsible for the color of the flower, and they also protect plants against microorganisms and insects. Flavonoids are attractants to pollinators. It protects plants against harmful ultraviolet radiation and helps signal molecules to facilitate nitrogen fixation [27, 28]. They are well recognized for their antioxidant effects, protecting the body from various diseases [29]. The presence of a varied degree of flavonoids in different extracts of *A. precatorius* and *A. gangetica* confirmed the antioxidative effects with various health beneficiary aspects.

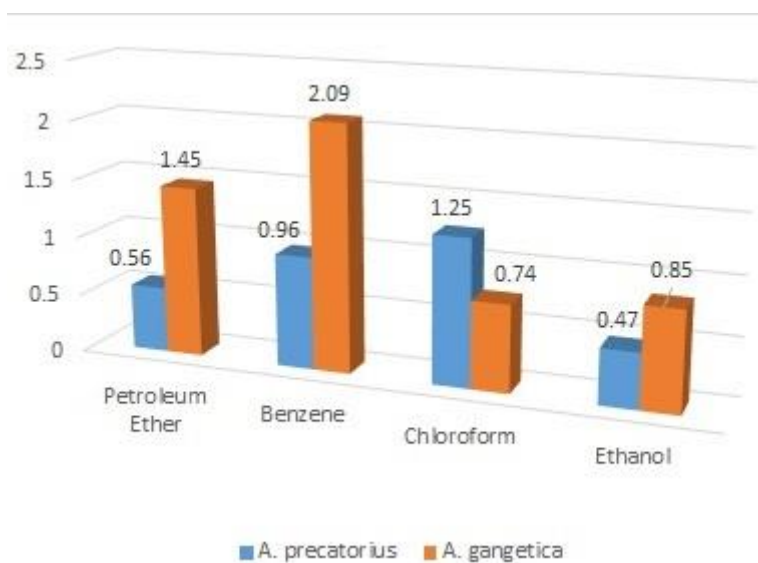
The reduction of Mo (VI) to green phosphate in phosphomolybdenum is due to antioxidant compounds. The results of phosphomolybdenum assay of *A. precatorius* and *A. gangetica* extracts are illustrated in Table 2. Among the various extracts of *A. precatorius*, ethanolic extracts showed the strongest phosphomolybdenum reduction (65.9 g AA/100 g), followed by petroleum ether extracts (60.3 g AA/100 g). Chloroform and benzene extracts showed the minimum level of phosphomolybdenum reduction in *A. precatorius*. In *A. gangetica*, ethanolic extracts registered the highest phosphomolybdenum reduction (53.9 g AA/100 g), followed by a reasonable reduction of benzene extract (21.0 g AA/100 g). Chloroform and petroleum ether extracts demonstrated a minor level of reduction. Phosphomolybdenum assay is very simple and independent of other antioxidant assays due to its varied application to plant extracts [18]. Electron transfer from antioxidants to Mo (VI) complex occurs in phosphomolybdenum assay. This reduction ability was observed in extracts of *A. precatorius* and *A. gangetica*.

The nitric oxide radical scavenging activity was determined using Griess reagent. The amount of nitrous acid will decrease in the presence of a plant extract, and it can be measured at 546 nm. The nitric oxide radical scavenging activity of different extracts of *A. precatorius* and *A. gangetica* is shown in Table 3. In *A. precatorius*, higher NO radical scavenging activity was observed in benzene extracts (87.9%), followed by chloroform and ethanolic extracts (82.0 and 67.4%), respectively. Petroleum ether extracts showed the lowest percentage (53.4%) of



free radical scavenging activity in *A. precatorius*. The ethanolic extracts of *A. gangetica* exhibited a maximum percentage (92.2%) of NO radical scavenging activity, followed by chloroform and benzene extracts (81.5 and 75.2%, respectively). The lowest percentage (55.3%) was observed in petroleum ether extracts of *A. gangetica*. The results indicate that all the extracts are expected to have this ability. Nitric oxide is formed from L-arginine by the activity of nitric oxide synthase. Nitric oxide depletes the concentration of ascorbic acid and initiates lipid peroxidation when it is exposed to human blood plasma. Nitric oxide is also responsible for cancer, inflammation, and various pathological conditions [30]. Jung *et al.* (2009)[31] performed antioxidant activity using nitric oxide assay in *Brassica juncea*, and the results showed noticeable activity against the radical. Similar to the previous observations, the inhibition shown by the benzene extracts of *A. precatorius* and ethanolic extracts of *A. gangetica* can have a significant role in scavenging nitric oxide radical.

The extracts of *A. precatorius* and *A. gangetica* showed an efficient H<sub>2</sub>O<sub>2</sub> scavenging potential (Table 4). Among the extracts of *A. precatorius*, ethanolic extracts showed maximum inhibition (96.8%), followed by petroleum ether and chloroform extracts (94.8 and 90.8%, respectively). In *A. gangetica*, petroleum ether extracts exhibited the highest inhibition percentage (96.3%), followed by chloroform and benzene extracts (88.3% and 81.7%), respectively. The results of H<sub>2</sub>O<sub>2</sub> scavenging activity indicate that the extracts could act as an effective scavenger of hydrogen peroxide. H<sub>2</sub>O<sub>2</sub> can be converted to a more reactive hydroxyl radical, which is one of the unfavorable effects caused by it [32]. The results showed that *A. precatorius* and *A. gangetica* could act as a good scavenger of hydrogen peroxide.



**Figure 1.** Reducing power activity of *A. precatorius* and *A. gangetica*.

The reducing property is related to the presence of reductants. The antioxidant property of reductants is based on the breakage of the free radical chain by donating a hydrogen atom. The reducing power of different extracts of *A. precatorius* and *A. gangetica* was illustrated in Fig. 1. A strong reducing power was observed in benzene extracts of *A. gangetica* followed by petroleum ether extract. In *A. precatorius*, maximum reducing power was seen in chloroform extracts. The data presented here clearly indicate the marked reducing potential of the tested extracts due to the presence of phenolic compounds, which act as reductants by donating the electrons. It reacts with free radicals and terminates radical chain reaction.

Pal *et al.* (2009) [33] screened the seed extracts of *A. precatorius* for their potential antioxidant activities using hydroxyl radical, reducing power and hydrogen peroxide scavenging activity. The results showed potent antioxidant activity when compared with the reference compound butylated hydroxyl toluene (BHT). Akula and Odhav (2008)[34] studied the DPPH radical scavenging activity of *A. gangetica* and found that methanolic extracts were more effective DPPH radical scavengers than the aqueous extracts. Reddy *et al.* (2012) [35] studied the methanolic extract of *A. gangetica* for potent antioxidant properties using different *in vitro* models. It possesses potent DPPH radical scavenging activity and reducing power effects. Similar to the previous observations, the present study results depicted the free radical scavenging properties of different extracts of *A. precatorius* and *A. gangetica*, which may be due to the presence of phenols and flavonoids.

#### 4. Conclusion

The results showed a clear indication that the extracts of *A. precatorius* and *A. gangetica* have widespread use as antioxidants. These findings also justify that these plants are an easily accessible source of natural antioxidants, which can be used as a dietary supplement in nutraceutical and pharmaceutical products. Further studies are needed to isolate the bioactive compounds from these plants, and it will surely pave the way for promoting natural drugs for treating diseases.

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#### Conflicts of Interest

The authors declare no conflict of interest.

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