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Original article

Phytochemical analysis and comparative study of *in vitro* free radical scavenging activity of different extracts of leaves of *Abrus precatorius* L.

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Abstract

The present study was carried out to evaluate and find the extracts having potential *in vitro* antioxidant property of leaves of *Abrus precatorius* L. (Family: Fabaceae). The free radical's scavenging effect was determined by *in vitro* antioxidant assays, using 2, 2-diphenyl-2-picryl-hydrazyl free radical (DPPH) method. The extract was obtained by maceration process using three solvents, namely; water, methanol and chloroform. The phytochemical analysis of the leaves revealed the presence of alkaloids, saponin, tannin and several polyphenols. The different concentrations of extracts (µg/ml) were prepared and used to evaluate antioxidant activity as a percentage inhibition. The results showed that percentage inhibition of DPPH by methanol, chloroform and aqueous extracts were 75.16, 72.09 and 62.22% at 300 µg/ml, respectively. Methanolic extract of *A. precatorius* showed good inhibition as compared to ascorbic acid ($p<0.05$) due to high phenolic content in the leaf extract. It was concluded that methanolic extract of leaves of *A. precatorius* plant exhibited potent *in vitro* free radical scavenging activity.

Key words: *Abrus precatorius* L., phytochemical analysis, antioxidant activity

1. Introduction

Abrus precatorius L. is commonly found in the tropical and sub-tropical regions of the World. It is native to India. It belongs to Febaceae family, commonly known as Gunja or Jequirity (John *et al.*, 2012). The stem, bark, leaves and roots of this plant have medicinal potential to cure and prevent various disease conditions. The different parts of the plant are also used as a traditional medicine to treat scratches, sores and wounds caused by animals and mechanical injury (Attal *et al.*, 2010). The leaves of *A. precatorius* have laxative, expectorant and aphrodisiac properties and are used to cure fever, cough and cold (Pade, 1957). Several active compounds have been isolated and identified from the leaves of *A. precatorius* including abrine, trigonelline, abruslactone A, hemiphloin, abrusoside A, abrusoside B, abrusoside C, abrusoside D, arabinose, galactose, xylose, choline, hypaphorine, precatorine (Garaniya and Bapodra, 2014).

Free radicals or reactive oxygen species (ROS) are produced by xenobiotics in living organism which may destroy the physiological process at cellular level (Madhavi *et al.*, 1996). Oxidative stress produced by increasing of reactive oxygen species is associated with various disease conditions. Oxidative stress causes the loss of physiological functions of tissues and organs (Fang *et al.*, 2002).

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There is crucial role of medicinal plants in human society and animal well being to combat diseases (Nostro *et al.*, 2000). India is commonly found of huge of herbal plants. Nowadays, various diseases have been treated by medicinal plants (Raghavendra *et al.*, 2013). Medicinal plants are widely used for the treatment of various diseases due to presence of active phytoconstituents. Phytoconstituents of plants having antioxidant properties which can prevent various biochemical processes. Antioxidant properties are also used for potential therapeutic effect. Natural antioxidant effects of compounds derived from medicinal plants are most popular nowadays (Pal *et al.*, 2009; Rajeshwari *et al.*, 2013; Manoharachary and Nagaraju, 2016). There have many unknown chemical substances in medicinal plants which are produced therapeutic effects. The leaves extract of *A. precatorius* have been shown to possess antibacterial, antifungal (Prashith *et al.*, 2010), antitumor, immunopotentiating (Ghosh and Maiti, 2007) and anti-inflammatory activities (Anam, 2001). Based on the traditional uses and pharmacological properties of *A. precatorius*, the present study was conducted to investigate *in vitro* antioxidant activity of the different extracts of leaves of *A. precatorius*.

2. Materials and Methods

2.1 Plant material

Leaves of *A. precatorius* L. were collected from surrounding of Junagadh district and authenticated by Mr. Punit Bhatt of this department. The voucher specimen (No. JVC/VPT/SP/24/2018) has been deposited in the herbarium of the department. The leaves were cleaned under running tap water and shed dried in laboratory.

The leaves powder was made using mixture grinder and stored in air tight container.

2.2 Preparation of extract

Different extracts of the plant were prepared by maceration process. 10 g of plant material was uniformly soaked in 100 ml. chloroform, water and methanol for 72 h, followed by filtration of extracts with the help of filter paper (Whatman No. 1). The solvent was evaporated in a rotary evaporator. The dried extracts were used to evaluate *in vitro* antioxidant activity.

2.3 Chemicals and reagents

Chemical such as 2, 2-diphenyl-1-picryhydrazyl (DPPH) was procured from Sigma Aldrich, Bengaluru, India. L-ascorbic acid and solvents of analytical grade were procured from Merck Ltd. Mumbai, India.

2.4 Phytochemical screening of leaves of *A. precatorius*

Chemical tests were performed for different extracts according to standard methods to detect major phytochemicals like alkaloids, glycosides, saponins, phenol, tannins, *etc.*, in the extract (Evans, 2002).

2.4.1 Detection of alkaloids

Methanolic extract was diluted in acidic solution like 1-5% HCL. This test solution was used for detection of alkaloid using various reagents.

Dragendorff's test: 1 ml of test solution was mixed with Dragendorff's reagent. Formation of bright orange precipitates confirmed presence of alkaloid in the sample.

Mayer's test: 1 ml of test solution was mixed with Mayer's reagent. Formation of white or buff precipitates confirmed presence of alkaloid in the sample.

Wagner's test: 1 ml of test solution was mixed with Wagner's reagent. Formation of brown precipitates confirmed presence of alkaloid in the sample.

Hager's test: 1 ml of test solution was mixed with Hager's reagent. Formation of yellow precipitates confirmed presence of alkaloid in the sample.

2.4.2 Detection of flavonoids

Extract dissolved in methanol solvent can be used as test solution.

Shinoda test: 1 ml of test solution was mixed with magnesium powder and a few drops of concentrated HCL was added. Development of orange, pink, red to purple color which showed the presence of flavonoid. By using zinc instead of magnesium, only development of deep-red to magenta color or weak pink to magenta color or no color which indicates presence of flavonoid.

Sulfuric acid test: 1 ml of test solution was mixed with few drops of concentrated H_2SO_4. Flavones or flavonols, chalcones or aurones and flavanones produced a deep yellow, red bluish and orange to red colors solution, respectively.

2.4.3 Detection of sterol

Extract dissolved in chloroform solvent can be used as test solution.

Salkowaski test: 2 ml of concentrated H_2SO_4 was added into 1 ml of test solution along the side of the test tube, forming two phases and development of red color which indicates the presence of sterol.

2.4.4 Detection of phenols

Ferric chloride test: Test solution was mixed with 1 ml of 5% $FeCl_3$ in 90% methanol. It was observed for blue, blue-black, or blue-green color which indicates the presence of polyphenols.

2.4.5 Detection of saponins

Pinch of extract was dissolved in water and shaken well. Formation of foam indicated the presence of saponin in the test sample. Foam which was stable for 15 min or more, then it was considered as positive.

2.4.6 Detection of sugars

Extract dissolved in polar solvent like water can be used as test solution.

Molisch's test: 1 ml test solution was mixed with 1 ml of 10 % methanolic α-napthol solution and then 4 to 5 drops of concentrated H_2SO_4 was added along the side of the test tube. It was observed for violet ring which indicates the presence of glycoside or sugar.

2.5 Determination of antioxidant activity

The scavenging activity of various extracts of *A. precatorius* leaves was determined using DPPH assay (Senguttuvan *et al*., 2014). One mg per ml solution was prepared by dissolving 30 mg extract in 30 ml Milli-Q water. Suitable dilutions were made from this stock solution with Milli-Q water only. All the dilutions were taken in test tubes up to 3 ml of sample solution of different concentrations (15, 30, 75, 150, 225, 300 µg/ml). 4 mg of DPPH was dissolved into 100 ml methanol to prepare 0.1 mM DPPH solution. Then 3 ml of sample solution was mixed with 1 ml of 0.1 mM DPPH. The reaction mixture was incubated for 20 min at 28°C in the dark. The control containing 3 ml water and 1 ml 0.1 mM DPPH was used as blank. Then, absorbance of sample solution of varying concentrations was taken. The DPPH radical scavenging activity was determined by measuring the absorbance at 517 nm, using a spectrophotometer (UV-2900). Standard ascorbic acid of various concentrations like 15, 30, 75, 150, 225, 300 µg/ml were prepared into water and served as a positive control. The assay was performed in triplicate. The antioxidant activity of plant extracts were calculated using the following equation which expressed as percent inhibition.

%inhibition = (Absorbance of control – Absorbance of control)/ Absorbance of control ×100

Statistical analysis: All data were expressed in Mean ± S.E. (n=3). Data were analyzed by one-way ANOVA followed by Duncan Multiple Range Test (DMRT) to compare difference in means.

3. Results and Discussion

3.1 Phytochemical analysis of leaf extracts of *A. precatorius*

Presence of various qualitative phytochemicals screening of *A. precatorius* leaf extract is shown in Table 1. Various disease conditions protected by phytochemicals defense mechanism due to the presence of terpenoid, alkaloids, saponin, tannin and phenolic

compounds. In the present study, phytochemical analysis has been performed in chloroform, water and methanol extracts of *A. precatorius* leaf which revealed the presence of alkaloids, saponin, tannin and flavonoids. Methanolic extracts of leaf have been observed major phytochemicals like, alkaloids, tannin and flavonoids as compared to chloroform and water extracts. Methonolic solvent has subjected to extract major phytochemicals due to the soluble of the wide range of polar substances. This result supported with previous reported studies on methanolic extract of leaf of *A. precatorius* (Georgewill and Georgewill, 2009; Kuo *et al.*, 2009). Presence of flavonoids in *Nyctanthes arbortristis* L. (leaf) (Ladumor *et al.*, 2017) and *Argyreia osyrensis* (leaves and fruits) (Mahendra *et al.*, 2016) and *Aegle marmelos* (Tiwari *et al.*, 2016) are reported earlier. Many flavonoid and other secondary metabolites from crude extract of plant showed effective hepatoprotective, antifungal, antipyretic, antibacterial, anti-inflammatory and antioxidant activities. These results agreed with Taur and Patil (2005) and Gnanavel and Mary (2013) who have observed that the ethanolic and petroleum ether extracts of *A. precatorius* leaves showed presence of steroids, saponins, alkaloids, flavonoids and glycoside.

Test for alkaloids: Samples were tested with Wagner's test, Hager's test, Mayer's test and Drangendorff's test for detection of alkaloids. Chloroform and water extracts did not produce in color in all tests which indicate of absence of alkaloids. However, methanol extract showed change in colors in Mayer's test and Drangendorff's test which indicates that methanol extract of plant contains alkaloids.

Test for flavonoid: Shinoda test with magnesium metal, Shinoda test with zink metal and Sulfuric acid test for flavonoid were detected in extract samples. Water and methanol extracts were observed color in Shinoda test with magnesium metal and Sulfuric acid test which showed presence of flavonoid in samples, while chloroform did not produce in color in all tests which indicated of absence of flavonoid. Flavonoids exhibits numerous pharmacological properties like gastroprotective, antisecretary, cytoprotective and antioxidant activities (Narayana *et al.*, 2001). Likewise, the fraction of the flavonoid extract proved gastric protection (Kuhnau, 1976).

Test for saponin: Water extract was observed the stabilize foaming up to 10 min, which was an indication of presence of positive in samples. When chloroform and methanol extracts were not showed foam.

Test for sterol: Salkowaski's test showed brown color in methanol extracts, while chloroform and methanol extracts were observed colorless which indicated the absence of sterol in samples.

Test for sugars: Red brown ring was observed in water and methanol extracts treated with Molisch's test while chloroform extract has no color change which indicated the negative result of samples

Test for tannins: All extracts treated with ferric chloride test were observed color change which was indicated the positive result of samples.

3.2 Antioxidant activity of leaf of *A. precatorius*

Preliminary free radical activity of the plant extract was evaluated by DPPH scavenging activity (Bhuiyan *et al.*, 2009). The DPPH assay is rapid, reliable and reproducible which is commonly used for the evaluation of *in vitro* antioxidative properties of pure compounds as well as plant extracts (Koleva *et al.*, 2002). The reducing capacity of test compounds can serve as indicator of potential antioxidant property (Meir *et al.*, 1995). Plant extracts acts as electrons donor, were determined by *in vitro* antioxidant assays, using 2, 2-diphenyl-2-picryl-hydrazyl free radical (DPPH) scavenging because of their content in phenolic compounds (Braca *et al.*, 2002).

Table 1: Phytochemical screening of various extracts of *A. precatorius* leaves

Phytochemicals	Extracts	Results
Test for alkaloid		
A. Mayer's test	Water	Negative
	Methanol	Positive
	Chloroform	Negative
B. Drangendorff's test	Water	Negative
	Methanol	Positive
	Chloroform	Negative
C. Wagner's test	Water	Negative
	Methanol	Negative
	Chloroform	Negative
D. Hager's test	Water	Negative
	Methanol	Negative
	Chloroform	Negative
Test for flavonoid		
A. Shinoda'stest with magnesium metal	Water	Positive
	Methanol	Positive
	Chloroform	Negative
B. Shinoda'stest with zink metal	Water	Negative
	Methanol	Negative
	Chloroform	Negative
C. Sulfuric acid test	Water	Positive
	Methanol	Positive
	Chloroform	Negative
Test for saponin		
	Water	Positive
	Methanol	Negative
	Chloroform	Negative
Test for sterol		
Salkowaski's test	Water	Negative
	Methanol	Positive
	Chloroform	Negative
Test for sugars		
Molisch's test	Water	Positive
	Methanol	Positive
	Chloroform	Negative
Test for tannins		
Ferric chloride test	Water	Negative
	Methanol	Positive
	Chloroform	Positive

Table 2: DPPH scavenging activity of various extracts of *A. precatorius* leaves

Concentrations (µg/ml)	Percent inhibition (mean ± SE)			
	Ascorbic acid	Methanol	Chloroform	Water
15	57.44 ± 1.89^{b}	47.49 ± 5.27^{a}	43.85 ± 5.50^{a}	43.53 ± 7.55^{a}
30	58.33 ± 2.25^{b}	51.05 ± 5.66^{ab}	47.65 ± 6.54^{a}	50.73 ± 10.50^{ab}
75	59.55 ± 2.14^{a}	55.18 ± 5.30^{a}	52.99 ± 5.68^{a}	53.88 ± 8.96^{a}
150	63.83 ± 2.16^{a}	64.81 ± 4.95^{a}	60.03 ± 6.73^{a}	59.63 ± 9.44^{a}
225	64.56 ± 1.85^{ab}	71.28 ± 5.19^{b}	64.64 ± 6.41^{ab}	58.25 ± 10.23^{a}
300	65.45 ± 1.86^{a}	75.16 ± 3.39^{b}	72.09 ±7.53^{b}	62.22 ± 7.45^{a}

Values in the same row with different superscripts are significantly (p<0.05) different.

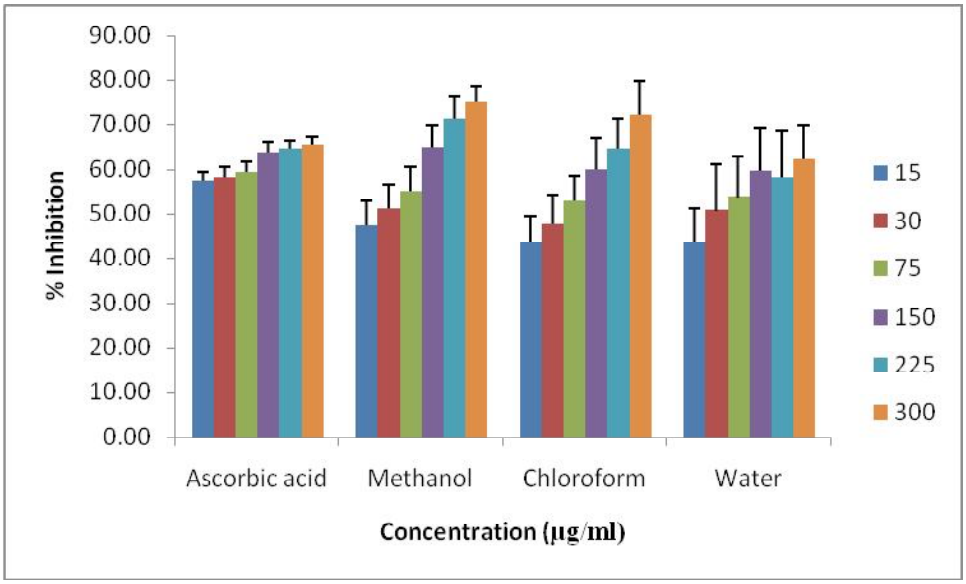

Figure 1: Percent inhibition of different extracts of *A. precatorius* leaves.

The antioxidant potential of various extracts of *A. precatorius* was determined by their ability to reduce the DPPH. Antioxidant activity was investigated by applying different concentrations (15, 30, 75, 150, 225 and 300 µg/ml) of leaves extracts of *A. precatorius*. The antioxidant activity of the different extracts is represented in Table 2.

In the present study, the percentage of scavenging effect was concomitantly increased with the increasing concentrations (15 to 300 µg/ml) of leaves extracts of *A. precatorius*. This result supports with previous study which demonstrated antioxidant properties of extracts from leaves of *A. precatorius* increased with increasing concentrations (Mir *et al.*, 2013). The inhibition ranges were from 47.49 to 75.16, 43.85 to 72.09 and 43.53 to 62.22 % for the methanol, chloroform and water extracts, respectively as compared to 57.44 to 65.45 % for the ascorbic acid. The methanol extract from the leaves of *A. precatorius* produced significantly (p<0.05) higher inhibition of DPPH at 225 µg/ml concentration as compared to the chloroform and water extracts. Moreover, it was observed that chloroform extract has produce marked scavenging activity equivalent with methanolic extract at 300 µg/ml concentration.

This effect might be due to effective hydrogen donating capability of methanolic extract leaves of *A. precatorius*, which indicates good source of antioxidants (Michalak, 2006). These results were in accordance with Repetto and Liesuy (2002) who had observed that the antioxidant activity of flavonoids is efficient in trapping super-oxide anion, hydroxyl, peroxyl and alcohoxyl radicals. Flavonoids have been reported as strong antioxidant bioactive compounds against free radicals which act on number of hydroxyl substitutions (Pandey and Rizvi, 2009). In our study, leaf extracts of *A. precatorius* showed significant scavenging activity against free radicals which may be due to the presence of phenolic compounds. Present finding is supported by Mahendra *et al.* (2016) and Kuntal *et al.* (2017) who have reported that the presence of phenolic compounds including flavonoids which are responsible for antioxidants activities and reducing power of plant extract.

4. Conclusion

In present study, different extracts of leaves of *A. precatorius* contain diverse phytochemicals especially the alkaloids, flavonoids, tannin and others that are responsible for higher free radical scavenging activity by DPPH. The major phytochemicals have been confirmed

to present from the methanolic extract of the leaves of *A. precatorius* by chemical methods analysis. The presence of secondary metabolites having pharmacological properties can be used of the leaves of *A. precatorius* for various ailments by traditional practitioners. The leaves of *A. precatorius* showed radical scavenging activity in all the solvent (chloroform, water and methanol) extracts. Out of them, methanolic extract from *A. precatorius* leaves produced very good inhibition against free radicals which might be due to high phenolic content. Therefore, it can be used as a source of natural antioxidants.

Conflict of interest

We declare that we have no conflict of interest.

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