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PHYTOCHEMICAL SCREENING, ANTHELMINTIC AND ANTIOXIDANT POTENTIAL OF *DIPCADI KRISHNADEVARAYAE* (ASPARAGACEAE)

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ABSTRACT

Dipcadi krishnadevarayae (Asparagaceae) is a bulbous monocotyledonous medicinal plant discovered in 2016 in different localities of Ananthapuramu district, Andhra Pradesh. In the present work, we studied its anthelmintic and antioxidant potential of methanolic and n-hexane extracts. Phytochemical investigations revealed that methanolic extract of both whole stem and bulbous parts possess flavonoids, cardiac glycosides, alkaloids, saponins and tannins. Significant anthelminthic activity was found with methanolic stem extract against Standard drug Albendazole. Antioxidant potential was performed by Folin-Ciocalteu's method, Free Radical Scavenging Activity (FRSA), UV spectrophotometric method and Oyaizu method and the results revealed that both methanolic stem and bulb extracts possess considerable antioxidant activity when compared with standard antioxidants.

KEY WORDS

Dipcadi krishnadevarayae, Phytochemical studies, antioxidant activity, anthelminthic activity, Albendazole.

INTRODUCTION

Dipcadi Medik. comprising 41species [1] belongs to tribe Ornithogaleae, subfamily Scilloideae and family Asparagaceae [2]. In India, the genus is recorded with 11 species, of which 9 are endemic to the country and these are distributed in from Himalayas to Peninsular India. *Dipcadi krishnadevarayae* B.R.P. Rao, the candidate species of the present phytochemical investigation is discovered in 2016 in different localities of Ananthapuramu district, Andhra Pradesh. It is a perennial scapigerous herb with ovoid-globose bulbs of 4.5 x 3.5 cm size and differs from all the other species of *Dipcadi* in having longer scapes (c. 85 cm), 20-40 cm long racemes, distinctly 6-lobed stigma and number 5-10 seeds per locule as shown in Fig 1.

Phytochemicals are the chemicals produced by plants through primary and secondary metabolism and generally have biological activity in the plant host and play a key role in plant growth and defense mechanisms. Flavonoids are widely distributed in plants in the form of polyphenolic compounds. They impart antioxidant, antimicrobial, anticancer and *etc*. activities to the plant. Among all, antioxidant activity is the therapeutically important as oxidative stress leads to several health disorders like myocardial infarction, hepatotoxicity and different forms of cancers. Flavanoids exhibit free scavenging activity and prevents tissue oxidation due to accumulation of super oxides [7]. Generally, tannins and saponins being bitter principles elicit anthelminthic activity.

As some of the *Dipcadi* species revealed the presence of alkaloids, tannins, saponins and flavonoids [3, 4, 5, 6], we attempted to study the phytochemistry of *Dipcadi krishnadevarayae*. Since these compounds have anthelminthic and antioxidant activity, the present work is emphasised on phytochemical, anthelminthic and

antioxidant activity evaluation for methanolic and n-hexane extract of whole stem and bulbous parts of the species. Experimentation methods, and the results obtained in preliminary phytochemical screening and the chemical compounds antioxidant and anthelminthic activity is discussed.

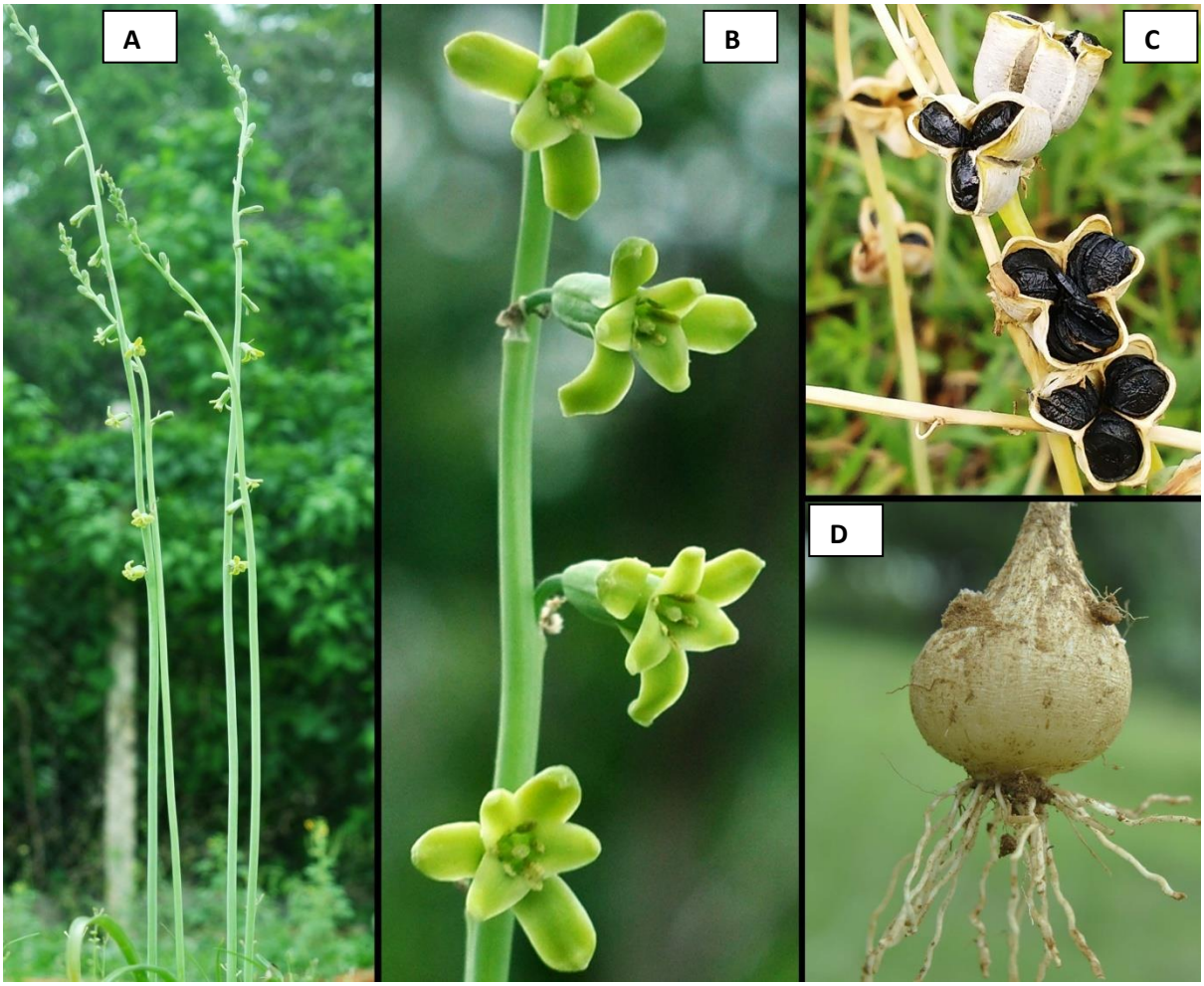


Fig. 1: *Dipcadikrishnadevarayae*A. Habit B. Flowers C. Seeds D. Bulb

MATERIALS & METHODS

MATERIALS

Collection of plant material

The whole plants of *Dipcadi krishnadevarayae* was collected from a rocky terrain along road sides in the outskirts of Garladinne Village, Ananthapuramu district, Andhra Pradesh, India. Voucher specimens were deposited in S.K. University Herbarium (SKU) (Acc. No. 51138), Ananthapuramu.

Chemicals

Folin-ciocalteu'sreagent, DPPH was purchased from Sigma-Aldrich (Bangalore, India).

METHODS

Preparation of extracts

The dried and powdered whole plant of *Dipcadi krishnadevarayae* was passed through a sieve no.22 and each 500 grams of powder were extracted successively by cold percolation [8] with one litre of n-hexane and methanol respectively. The extracts were concentrated to dryness under reduced pressure using rotary vacuum evaporator and used for further investigations.

Chemical tests

The methanolic and n-hexane stem and bulb extract solutions of *Dipcadikrishnadevarayae*were subjected to the following chemical tests tabulated in Table 1.

Table 1: Chemical tests on methanolic and n-hexane extracts of *D. Krishnadevarayae*

Phytochemical constituent	Chemical test	Procedure
Alkaloids	Mayer's test	1 ml of solution + 0.5 ml Potassium mercuric iodide
	Dragendorff's test	1 ml of solution + 0.5 ml Potassium bismuth iodide solution
	Wagner's test	1 ml of solution + 0.5 ml Solution of iodine in Potassium iodide
	Hager's test	1 ml of solution + 0.5 ml Saturated solution of picric acid
	Tannic acid test	10% tannic acid solution
Glycosides	Legal's test	Extract was treated with pyridine and alkaline sodium nitroprusside solution
Saponin glycosides	Froth test	1 ml solution of drug in water in a semi microtube, shaken well and noted
	Hemolysis test	0.2 ml solution of sample (prepared in 1% normal saline) was added to 0.2 ml of blood in normal saline and mixed well. Centrifuged, red supernatant obtained was compared with control tube containing 0.2 ml of 10% blood in normal saline
Tannins and Phenolic compounds	Gelatin test	Extract was treated with 1% gelatin solution containing 10% sodium chloride
	Ferric chloride test	Extract was treated with Ferric chloride solution
	Alkaline reagent test	Extract was treated with sodium hydroxide solution
Flavonoids	Shinoda test	To the extract solutions few fragments of magnesium ribbon and HCl were added.
	Zinc-Hydrochloride reduction test	To the extract solutions a mixture of zinc dust and concentrated HCl were added.
	Alkaline reagent test	To the extract solutions, few drops of sodium hydroxide solution was added, an intense yellow colour was formed, discoloration was observed on addition of few drops of dilute acetic acid
Proteins	Millon's test	To the extract solutions 2 ml of Millons reagent (mercuric nitrate in nitric acid containing traces of nitrous acid), white precipitate appeared, turned red upon gentle heating.
	Ninhydrin test	Boiling of extract solutions with 0.2% solution of Ninhydrin.
Carbohydrates	Molisch's test	Extract solutions were treated with few drops of alcoholic alpha naphthol. 0.2 ml of Conc. sulphuric acid was added slowly through the sides of the test tube, purple to violet ring appeared at the junction
	Benedicts test	Extract solutions were treated with few drops of Benedicts reagent (alkaline solution containing cupric citrate complex)
	Fehlings test	Equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartarate and sodium hydroxide in distilled water) reagents were mixed and few drops of Extract solutions were added and boiled.
Terpenoids	Terpenoids test	Extract sample was taken in a test tube, then poured 10 ml of methanol in to it, shaken well and filtered to take 5 ml extract of sample. Then 2 ml of chloroform were mixed in extract sample and 3 ml of sulphuric acid were added. Formation of reddish brown colour indicated the presence of terpenoids.
Phosphate test	Ammonium molybdate test	Extract solution with Conc. Nitric acid solution
	Silver nitrate test	Extract solution with Silver nitrate
Sulphate test	Barium chloride test	Extract solution with Barium chloride, white ppt insoluble in hot water and hot conc. nitric acid
	Lead acetate test	Extract solution with Lead acetate

Identification of flavonoids by using TLC

In the present study, methanolic and n-hexane stem and bulb extract solutions of *Dipcadi krishnadevarayae* was subjected to Paper chromatography and TLC [9]. In this analysis chromatographic paper and aluminium plates (10 cm length) coated with silica gel were used anddifferent solvent systems were employed depending upon the nature of the analyte. For saturation of TLC chamber a sheet of filter paper which had soaked in the mobile phase [Toluene:Ethylacetate:Formicacid(36:12:5V/V)]was laid so as to cover internal part of three sides of the chamber. The chamber was left undisturbed to ensure saturation. A solution of stem and bulb extract solutionsof *D. Krishnadevarayae* was prepared in methanol and n-hexane. The spots of identical volume were applied 2cm away from the lower edge of the plate with the help of microcapillary tube. The solvent was allowed to evaporate after each application by air drying. The spotted plate was then placed vertically in the chamber with the bottom edge immersed in developing medium. The solvent system was allowed to run approximately up to8 cm then the plates were taken out and the solvent front was marked. The resolution of components of all extracts of the plant of *D. krishnadevarayae* was studied by locating the spots on the chromatogram. The spots were preliminarily identified by visual observation and then under UV lamp. Then the plates were developed in an Iodine chamber and the spots were located. The same procedure was used for n-hexane extract also. The spots were observed as prominent fluorescent green for stem and yellow for bulb extracts. In all the TLC images, left spot represents standard, whereas right spot, extracts.

Antioxidant activity

Determination of total phenolic content by Folin-Ciocalteu's method

The concentration of phenolics present in the methanolic and n-hexane stem and bulb extract solutions was determined using Folin-Ciocalteu's reagent spectrophotometrically. 1mg/ml methanolic and n-hexane stem and bulb extract solutions was used in the analysis. To the 0.5 ml of methanolic and n-hexane stem and bulb extract solutions, 2.5 ml of 10% Folin-ciocalteu's reagent which was dissolved in water and 2.5 ml of 7.5% sodium bicarbonate solution were added. Blank solution was also prepared in the same manner except addition of plant extract. The samples were thereafter incubated in a thermostat at 45ºC about 45 min. The absorbance was determined using spectrophotometer at 710 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of Gallic acid (Standard curve y = 7.012x – 0.0181, r^2= 0.999). The content of phenolics in the extract was expressed in terms of Gallic acid equivalent (mg of GAE/g of extract). The values obtained for the concentration of total phenols are expressed as mg of GAE/g of extract [10].

Determination of flavonoid content by UV spectrophotometric method

The content of flavonoids present in methanolic and n-hexane stem and bulb extract solutions was determined spectrophotometrically. One mg/ml methanolic and n-hexane stem and bulb extract solutions of *D. krishnadevarayae* were used in the analysis. To 1 ml of 1mg/ml sample solution 0.5 ml of 2% ethanolic aluminium chloride solution was added and the solution was kept at room temperature for 1 h. Blank solution was also prepared in the same manner excluding addition of plant extract. Then the absorbance was measured at 420 nm using UV spectrophotometer. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin (Standard curve y = 16.213x – 0.0581, r^2 = 0.999). The content of flavonoids in the extract was expressed in terms of rutin equivalent (mg of RUE/g of extract [10].

Reductive Ability

The Reductive ability of medicinal plants was determined according to the Oyaizu method [11]. Stem and bulb extracts of *Dipcadi krishnadevarayae* which were prepared using methanol and n-hexane were dissolved separately in 1ml of distilled water to this 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of Potassium ferric cyanide (1%) were added. The mixture was then centrifuged at 3000 rpm for 10 min. Then 2.5 ml of the upper layer of the solution was mixed with 2.5 ml of distilled water and 0.5ml of FeCl3 (0.1%). Absorbance was measured at 550nm. Butylated Hydroxyl Toluene (BHT) was used as reference compound. All the analysis was performed in triplicate. Reducing ability (%) was calculated according to formula..... (1).

$$= \frac{(V_0-V_1)}{V_0} \times 100$$

V_0= absorbance of control and the V_1= absorbance of the sample.

Free Radical Scavenging Activity (FRSA) using hydrogen peroxide

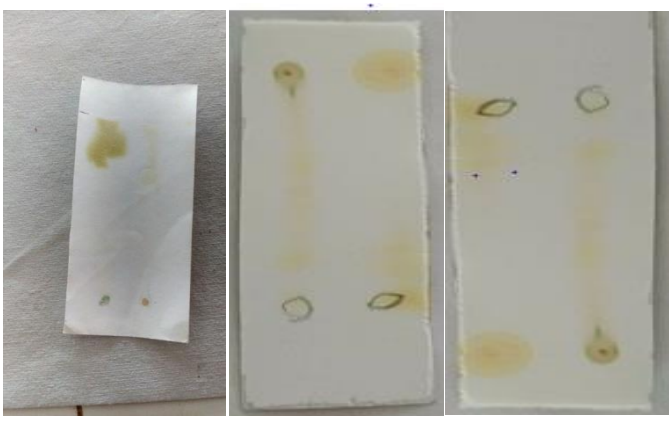
The hydrogen peroxide FRSA [12] of the methanolic and n-hexane stem and bulb extract solutions of *D. krishnadevarayae* was done as suggested by Czochra and Widwnsk. According to this method to 1.0 ml of methanolic sample (100 µg / ml), 2 ml of hydrogen peroxide (30 %) and 2.4 ml of 0.1 M phosphate buffer (p^H 7.4) were added. The resulting solution was kept for 10 min. The absorbance was recorded at 230 nm. All readings were repeated thrice. Blank was prepared without adding hydrogen peroxide and control was prepared without a sample. Ascorbic acid was used as a standard compound. Free radical scavenging activity of hydrogen peroxide (%) was calculated as per formula (1).

Anthelminthic activity

The Anthelminthic activity of methanolic and n-hexane extract solution of *Dipcadi krishnadevarayae* was carried on *Pheretima posthuma* (earthworms) [13]. Twenty ml of 50mg/ml, 100mg/ml and 150 mg/ml methanolic and n-hexane stem and bulb extract solutions of *D. krishnadevarayae* were prepared in distilled water and transferred into three different Petridishes containing 5 earthworms in each one. 20 ml of 10 mg/ml concentration Albendazole [14] was used as reference standard. Distilled water was used as the control. Movements of earthworms were observed to note paralysis and death time. Paralysis time was considered where the movements of earthworms were stopped and when earthworms showed no movement either by vigorous shaking or by sprinkling hot water on earthworms. The results obtained are tabulated (**Table 1**)

RESULTS AND DISCUSSIONS

Methanolic and n-hexane stem and bulb extract solutions of *Dipcadi krishnadevarayae* was found to possess antioxidant flavonoids as the chief constituents by chemical tests (Table 1) and assays. TLC Rf values were observed for both stem and bulb extracts between 0.5 to0.8 by using specific mobile phase which is nearer to the standard values (Albendazole) 0.3 to 0.8 as shown in Fig 2.



A B C

Fig. 2 TLC of A. Methanolic Stem extract B. Methanolic bulb extract C. n-hexane bulb extract

The total phenolic content in n-hexane stem and bulb extract solutions was found to be 20.5 ± 0.16 mg and 14.2 ± 0.28 GAE/g respectively and methanolic stem and bulb extract solutions was found to be 27.7 ± 0.12, 18.4 ± 0.1 mg GAE/g respectively. Total flavonoid content in n-hexane stem and bulb extract solutions was found to be 8.4 ± 0.13 and 4.8 ± 0.09 mg RUE/g respectively and methanolic stem and bulb extract solutions was found to be 12.7 ± 0.21 and 6.5 ± 0.09 mg RUE/g respectively. The antioxidant capacity of the extracts was performed by FRSA and Reductive ability methods. Results were observed as 89.57 % ± 0.230 % & 97.24 ± 0.150 for methanolic stem extracts and 75.67 % ± 0.320 % & 81.42 ± 0.370 for methanolic bulb extracts, 82.6 % ± 0.09 &

84.18 % ± 0.430 for n-hexane stem extracts and 79.6 % ± 0.19 & 74.27 % ± 0.340 for n-hexane bulb extracts respectively. Both methanolic and n-hexane extracts has shown considerable anthelminthic activity, results were tabulated in Table 2. However, bulb extracts have shown more anthelminthic activity when compared with stem extracts. No onset of paralysis and death was observed at 50 mg concentrations but 150 mg/ml solutions of both extracts has shown death of worms within 30 min.

Table 2: Qualitative analysis on phytochemical constituents

Chemical test	Methanolic extract of *D.krishnadevarayae*		n-hexane extract of *D. krishnadevarayae*	
	Stem	Bulb	Stem	Bulb
Mayer's test	+++	++	+	+
Dragendorff's test	+++	++	+	+
Wagner's test	+++	++	+	+
Hager's test	+++	++	+	+
Tannic acid test	+++	+	+	+
Legal's test	+++	++	+++	++
Froth test	-	-	-	-
Hemolysis test	++	++	++	++
Gelatin test	+	+	+	+
Ferric chloride test	+++	+++	+++	+++
Alkaline reagent test	+++	+++	+++	+++
Shinoda test	+++	++	+	+
Zinc-Hydrochloride reduction test	+++	++	++	+
Alkaline reagent test	+++	++	+	++
Millons test	++	-	+	-
Ninhydrin test	+	-	+	-
Molisch's test	++	++	++	++
Benedicts test	++	++	++	++
Fehlings test	++	++	++	++
Terpenoids test	+++	++	+++	++
Phosphates test	++	++	++	++
Sulphates test	++	++	++	++

+ + + :High presence of pyhtochemical constituents
+ + : Moderate presence of pyhtochemical constituents
+ : Low presence of phytochemical constituents
- : Absence of pytochemical constituents

Table 3: Anthelmentic activityof*Dipcadikrishnadevarayae*

Conc. (mg/ml)	Methanolic extract				n-hexane extract				Albendazole (10mg/ml)	
	Stem		Bulb		Stem		Bulb			
	PT (min)	DT (min)	PT (min)	DT (min)	PT (min)	DT (min)	PT (min)	DT (min)	PT (min)	DT (min)
50	40	ND	20	ND	40	ND	20	ND	5	15
100	30	45	15	30	30	45	15	20	-	-
150	15	30	15	20	15	30	10	20	-	-

PT- Paralysis time, DT- Death time, ND- No death, Min- Minutes

CONCLUSION

The phytochemical investigations on *Dipcadi krishnadevarayae* have proven that methanolic extracts of both stem and bulbous parts possess high presence of alkaloids, glycosides, inorganic ions like sulphates and phosphates. But maximum positive tests were observed with bulbous extracts for saponins and tannins. Flavonoids are associated in both stem and bulbous methanolic extracts and have significant antioxidant activity. Since saponins and tannins are present in high concentrations in bulbous extracts of both methanol and n-hexane they have shown potent anthelmenthic activity when compared with stem extracts. Having known the species anthelmintic and antioxidant

potential, we are keen and aimed to isolate and characterize the flavonoids present in *Dipcadi krishnadevarayae* and evaluating its anti-ancer and hepato-protective activities.

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