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EVALUATION OF *INVITRO* FREE RADICAL SCAVENGING ACTIVITY OF *CRINUM ASIATICUM* AND *PEDALIUM MUREX* LEAF EXTRACTS

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ABSTRACT: The aim of this research is to assess the *invitro* antioxidant activity of various leaf extracts of *Crinum asiaticum* and *Pedalium murex,* with a view to exploiting its potential as a source of natural antioxidants. DPPH assay i.e., 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) and Hydrogen peroxide assay was used for determination of free radical-scavenging activity of the ethanol, acetone and aqueous leaf extracts of *Crinum asiaticum* and *Pedalium murex.* The extracts were also studied for their phytoconstituents such as flavonoids, carbohydrates, tannins, saponins, proteins, alkaloids, phenols, terpenoids, fixed oils and glycosides. The phenolic concentration of the extracts was expressed as milligram of gallic acid equivalents per gram of extract. The ethanol and acetone leaf extracts of *Crinum asiaticum* and *Pedalium murex* showed the highest amount of phenolic compounds (77.3 mg/g and 82.2 mg/g) when compared to aqueous extract (36.65 mg/g). The antioxidant activity of *Crinum asiaticum* and *Pedalium murex* were evaluated using Vitamin E as standard. In our study, the ethanol and acetone leaf extracts of *Crinum asiaticum and Pedalium murex* exhibited appreciable scavenging activity when compared with aqueous leaf extracts. Based on the result in the study, it was concluded that ethanol and acetone leaf extracts of *Crinum asiaticum* and *Pedalium murex* were found to be a good natural antioxidant. Further studies are required to identify specific active principles of these plants for the significant antioxidant effect.

Keywords: Antioxidant activity, *Crinum asiaticum, Pedalium murex,* DPPH free radical scavenging assay, Hydrogen peroxide assay, Vitamin E.

I. INTRODUCTION

Phenolic compounds are commonly found in both edible and nonedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The importance of these antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists[1]. Flavonoids and other phenolics have been suggested to play a preventive role in the development of cancer and heart disease. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential.

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials as antioxidants. Various herbs and spices have been reported to exhibit antioxidant activity, including *Ocimum sanctum, Piper cubeba Linn., Allium sativum Linn., Terminalia bellerica, Camellia sinensis Linn., Zingiber officinale Roscoe* and several Indian and Chinese plants. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer[2]. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are included in various foods, but their safety, however, can be doubted[3]. For this reason, importance and demand for natural antioxidants has grown over the recent years. These antioxidants occur in all plants and in all parts of the plants[4]. It is already well known that the antioxidant activity shown by plants is mainly due to the presence of polyphenol components, which are secondary products from the plant metabolism[5].The initiation of lipid peroxidation is induced by the superoxide radical or by hydroxyl radicals. Therefore, antioxidation is an extremely significant action, which can be used as a preventive agent against a number of diseases[6, 7]. Polyphenols capture the free radicals by giving hydrogen atoms or electrons. Furthermore, their bioactivity may be related to the ability to chelate metals and inhibit lipooxygenases [8, 9].

Crinum asiaticum is a plant species widely planted in many warmer regions as an ornamental. It is a bulb forming perennial flowering plant. It is native of China, India, South Korea, Assam and Bangladesh belonging

to family Amaryllidaceae. *Pedalium murex* is a annual plant species widely planted in many warmer regions as an ornamental. It is a bulb forming perennial flowering plant. It is distributed in India, Srilanka and Tropical Africa belonging to family Pedaliaceae

So, an attempt was made to evaluate the antioxidant activity of various leaf extracts of *Crinum asiaticum* and *Pedalium murex*.

Fig:1 *Crinum asiaticum* plant

Fig:2 *Pedalium murex* plant

II. MATERIAL AND METHODS

2.1 Plant material

The plant was identified and authenticated by plant taxonomist Dr. K. Madhava Shetty, Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupathi, A.P, India. The plants were collected at Kapalitheertham forest A.P, India. The fresh leaves were separated from both the plants and shade dried. The shade dried leaves were powdered in a mechanical grinder and fine powder was collected by passing through sieve no: 40.

2.2 Preparation of leaf extracts

The powdered plant material 100g was extracted with water, ethanol and acetone separately using cold maceration method. The extracts were filtered with Whatman No.1 filter paper and the filtrates were concentrated in a vacuum evaporator. Dried extracts were used for further studies

2.3 Chemicals

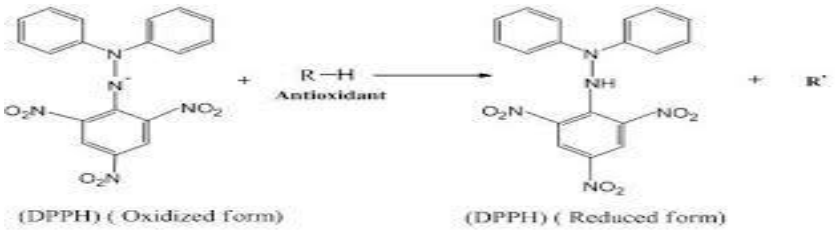
1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma (St. Louis,USA). Vitamin E, Potassium ferricyanide, ferric chloride, gallic acid, trichloroacetic acid and Folin-Ciocalteu reagent etc. were purchased from Merck India Ltd., Mumbai. All the chemicals and reagents used in the study were of analytical grade.

2.4 Phytochemical analysis and determination of total phenolic content

The extracts were studied for their phytoconstituents such as flavonoids, carbohydrates, tannins, saponins, proteins, alkaloids, phenols, terpenoids, fixed oils and glycosides using different phytochemical tests[10]. The total phenolic content of the aqueous, acetone and ethanol extracts were estimated by a colorimetric assay, according to the method described by Singleton and Rossi[11] with some modifications. Briefly, 1mL of sample was mixed with 1mL of Folin-Ciocalteu reagent. After 3min, 1mL of saturated Na_2CO_3 solution was added to the mixture followed by the addition of 7 mL of distilled water. The mixture was kept in the dark for 90 min, after which the absorbance was read at 725 nm. The total phenol contents were determined using a standard curve prepared with gallic acid. The estimation of the phenolic compounds was carried out in triplicate. The results were mean ± standard deviations and expressed as milligram of gallic acid equivalent/g of extract.

2.5 DPPH free radical scavenging assay

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the ethanol, acetone and aqueous leaf extracts. Briefly, 1.5mL of DPPH solution (0.004% in methanol) was incubated with 1.5mL of extracts at various concentrations (20-100 µg/mL). The reaction mixture was shaken well and incubated in the dark for 30min at room temperature. The control was prepared as above without extract. The absorbance of the solution was measured at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation[12,13]:



All determinations were performed in triplicate and calculated using the following equation:

% Scavenging activity = [1-Abs of sample (517nm) /Abs of control (517nm)] ×100

2.6 Hydrogen peroxide scavenging assay [14]

Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH 7.4). Different concentration of the extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of *Crinum asiacitum* and *Pedalium murex* of different leaf extracts and the standard were calculated by using formula:

$$\% \text{ Scavenged } [H_2O_2] = [(A_C - A_S) / A_C] \times 100$$

Where, A_C - Absorbance of the control

A_S - Absorbance in the presence of sample or standard

2.7 STATISTICAL ANALYSIS

Data are expressed as Mean ± SD . Statistical analysis was performed using one way ANOVA of three parallel measurements.

III. RESULTS AND DISCUSSION

1.1 Phytochemical analysis and determination of total phenolic content

The results of preliminary phytochemical screening of aqueous, acetone and ethanol leaf extracts of *Crinum asiaticum* and *Pedalium murex* in various leaf extracts was shown in Table 1 & 2.

Table 1: Phytochemical constituents of various leaf extracts of *Crinum asiaticum*

S.No.	Test	Ethanol	Acetone	Aqueous
1.	Flavonoids	++	++	+
2.	Carbohydrates	++	++	++
3.	Alkaloids	++	++	++
4.	Saponins	--	--	--
5.	Proteins	--	--	--
6.	Glycosides	--	--	--
7.	Phenols	++	++	+
8.	Fixed oils	+	+	--
9.	Tannins	--	--	--
	+ Presence of constituent		-- Absence of constituent	

Table 2: Phytochemical constituents of various leaf extracts of *Pedalium murex*

S.No.	Test	Ethanol	Acetone	Aqueous
1	Flavonoids	++	++	++
2	Carbohydrates	++	++	+
3	Alkaloids	++	++	+
4	Saponins	--	--	--
5	Proteins	--	--	+
6	Phenols	++	++	--
7	Fixed oils	--	--	++
8	Glycosides	--	--	--
9.	Tannins	--	--	--
	+ Presence of constituent		-- Absence of constituent	

3.2 Determination of total phenolic contents

The ethanol and acetone leaf extracts of *Crinum asiaticum* and *Pedalium murex* showed the highest amount of phenolic compounds (77.3mg/g and 82.2mg/g) when compared to aqueous extract (36.65mg/g). The phenolic concentration of the extracts was expressed as milligram of gallic acid equivalents per gram of extract. Phenols possess free radical scavenging ability due to their hydroxyl groups. The antioxidant action may be contributed by the phenolic compounds. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in human beings.

3.3 DPPH free radical scavenging activity & Hydrogen peroxide assay

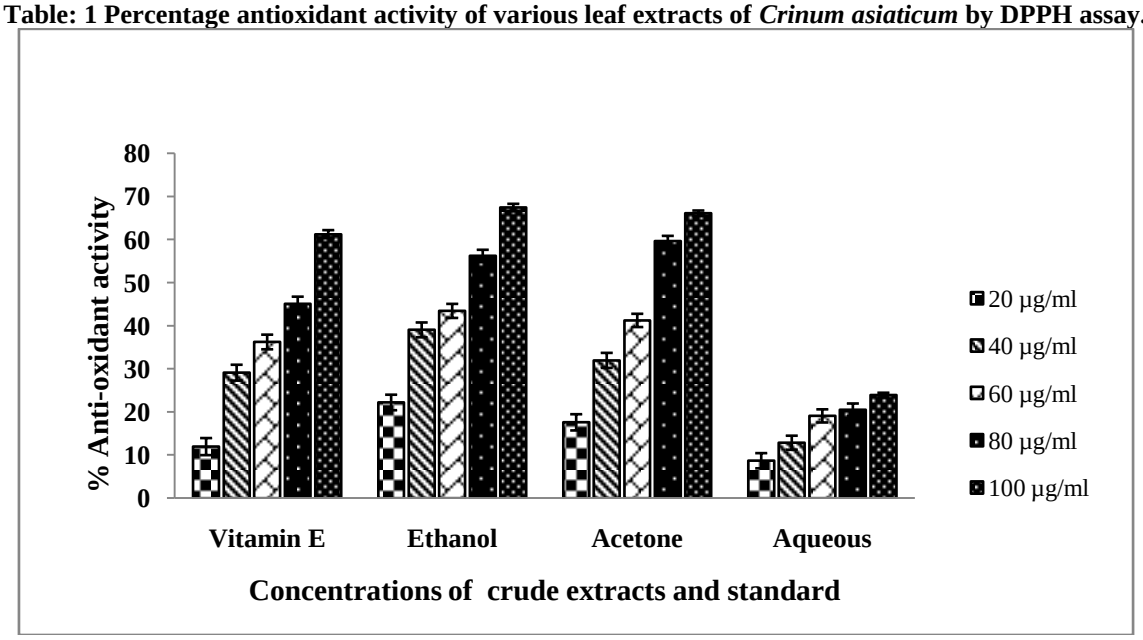
The anti-oxidant activity of different leaf extracts of *Crinum asiaticum* and *Pedalium murex* was evaluated by DPPH and Hydrogen peroxide assay. Vitamin E was used as positive control for investigation of anti-oxidant activity for both the assays. From the results, it was confirmed that ethanol and acetone extract of *Crinum asiaticum* and *Pedalium murex* has shown potent anti-oxidant activity.

The results of both the DPPH assay and Hydrogen peroxide assay of different leaf extracts of *Crinum asiaticum* were represented in table 1 & 2 and graphically represented in graph 1& 2 respectively.

The results of both the DPPH assay and Hydrogen peroxide assay of different leaf extracts *Pedalium murex* were represented in table 3 & 4 and graphically represented in graph 3 & 4 respectively.

S.No	Conc. µg/ml	% Scavenging (Mean ± SD) of Triplicates			
		Vitamin E	Ethanol	Acetone	Aqueous
1	20	15.97±1.98	8.92±1.8	14.6±1.88	6.9±1.76
2	40	33.12±1.84	17.5±1.72	29.97±1.74	11.87±1.63
3	60	46.24±1.7	26.95±1.63	43.42±1.54	19.09±1.52
4	80	52.09±1.66	39.42±1.42	52.63±1.23	24.53±1.44
5	100	61.25±0.94	54.62±0.87	63.09±0.65	42.92±0.54

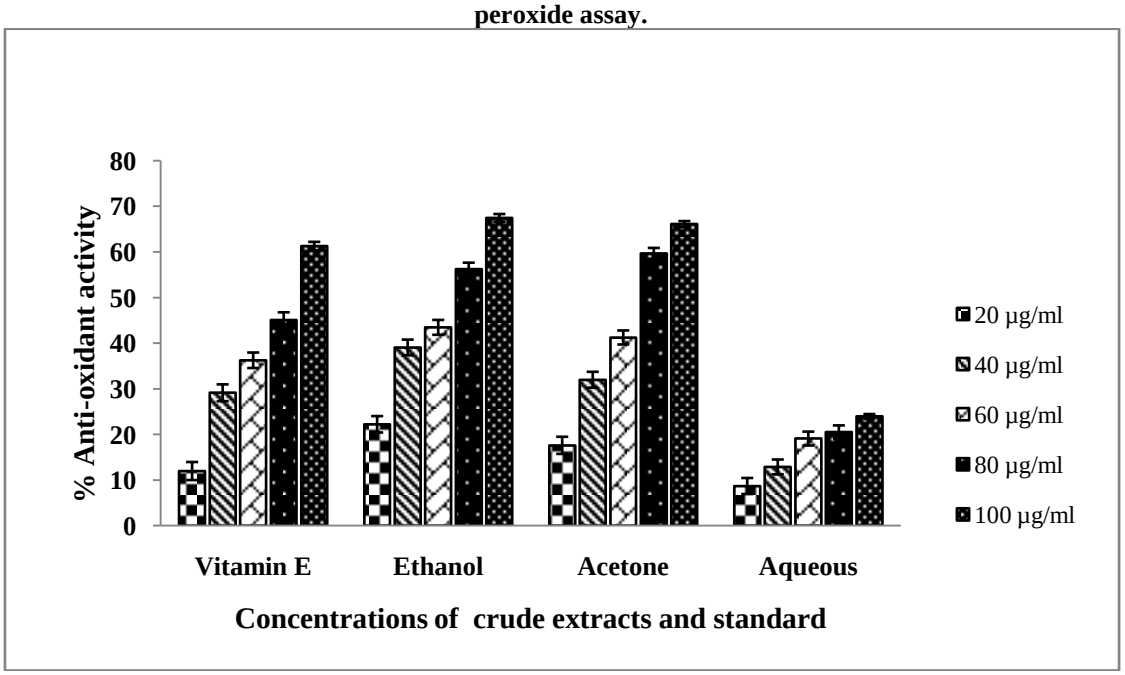
Table: 1 Percentage antioxidant activity of various leaf extracts of *Crinum asiaticum* by DPPH assay.



Graph: 1 Percentage antioxidant activity of various leaf extracts of *Crinum asiaticum* by DPPH assay.

S. No	Conc. µg/ml	% Scavenging (Mean ± SD) of Triplicates			
		Vitamin E	Ethanol	Acetone	Aqueous
1	20	11.97±1.89	9.92±1.9	16.6±1.98	8.9±1.96
2	40	29.12±1.4	19.5±1.72	29.97±1.74	19.87±1.63
3	60	36.24±1.5	26.95±1.63	43.42±1.54	19.09±1.52
4	80	45.09±1.8	39.42±1.42	52.63±1.23	24.53±1.44
5	100	61.25±0.94	55.62±0.87	69.09±0.65	32.92±0.45

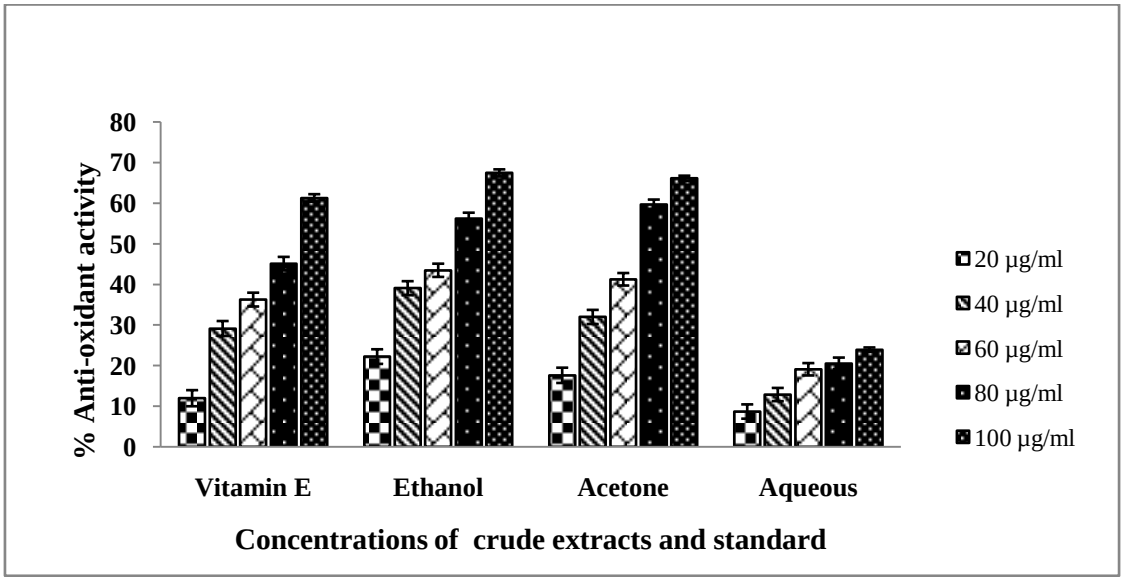
Table: 2 Percentage antioxidant activity of various leaf extracts of *Crinum asiaticum* by Hydrogen peroxide assay.



Graph: 2 Percentage antioxidant activity of various leaf extracts of *Crinum asiaticum* by Hydrogen peroxide assay

S.No	Conc. µg/ml	% Scavenging (Mean ± SD) of Triplicates			
		Vitamin E	Ethanol	Acetone	Aqueous
1	20	12.97±1.98	17.92±1.8	13.6±1.88	7.09±1.76
2	40	30.12±1.84	26.4±1.72	28.97±1.74	10.87±1.63
3	60	39.24±1.7	33.95±1.63	44.42±1.54	15.09±1.52
4	80	47.09±1.66	51.42±1.42	51.63±1.23	18.53±1.44
5	100	58.25±0.94	63.62±0.87	62.09±0.65	22.92±0.54

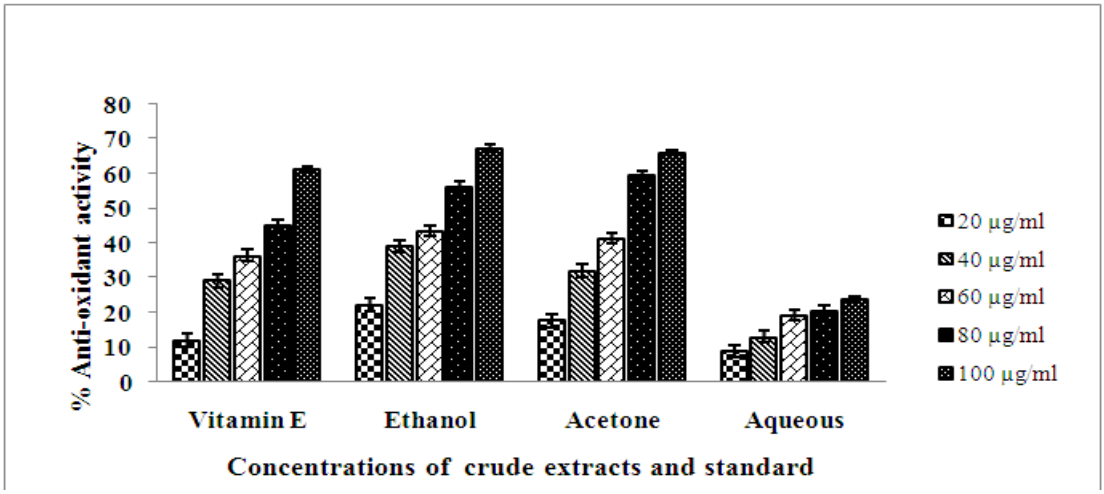
Table: 3 Percentage antioxidant activity of various leaf extracts of *Pedalium murex* by DPPH assay.



Graph : 3 Percentage antioxidant activity of various leaf extracts of *Pedalium murex* by DPPH assay.

S.No	Conc. µg/ml	% Scavenging (Mean ± SD) of Triplicates			
		Vitamin E	Ethanol	Acetone	Aqueous
1	20	11.97±1.98	22.21±1.8	17.6±1.88	8.9±1.76
2	40	29.12±1.84	39.05±1.72	31.97±1.74	12.87±1.63
3	60	36.24±1.7	43.45±1.63	41.42±1.54	19.09±1.52
4	80	45.09±1.66	56.21±1.42	59.63±1.23	20.53±1.44
5	100	61.25±0.94	67±0.87	66.09±0.65	23.92±0.54

Table: 4 Percentage antioxidant activity of various leaf extracts of *Pedalium murex* by Hydrogen peroxide assay.



Graph: 4 Percentage antioxidant activity of various leaf extracts of *Pedalium murex* by Hydrogen peroxide assay

IV. CONCLUSION

The antioxidant activity of *Crinum asiaticum* and *Pedalium murex* were evaluated using various antioxidant assays like DPPH radical scavenging and Hydrogen peroxide scavenging assay. In our study, the ethanol and acetone leaf extracts of *Crinum asiaticum* and *Pedalium murex* exhibited appreciable scavenging activity when compared with aqueous leaf extracts. Based on the result in the study, it was concluded that leaf extracts of *Crinum asiaticum* and *Pedalium murex* were found to be a good natural antioxidant. Further studies are required to identify specific active principles of this plant for the significant antioxidant effect.

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