INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

EVALUATION OF INVITRO ANTIOXIDANT ACTIVITY OF ETHANOLIC ROOT EXTRACT OF *CURCULIGO ORCHIOIDES*

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ABSTRACT

The present study was an endeavor to evaluate the antioxidant activity of ethanolic root extract of *Curculigo orchioides* which is commonly called as Golden eye grass(Fam:Hypoxidaceae).The Antioxidant potential of *Curculigo orchioides* was investigated by three different established invitro methods DPPH, Reducing Power and Phosphomolybdenum assay. Gallic acid was used as reference standard. The results obtained showed ethanolic root extract of *Curculigo orchioides* possess significant free radical, reducing power, antioxidant activity in a concentration dependant manner. The results revealed that ethanolic root extract of *Curculigo orchioides* possess significant antioxidant activity.

Keywords: *Curculigo orchioides*, DPPH, Reducing Power, PhosphoMolybdenum assay.

1. INTRODUCTION

Free radicals are produced by the body to aid in the metabolic processes, such as digestion[1].Free radicals are documented for playing a dual role in our body as both beneficial&delteriousspecies.Excess production of ROS (reactive oxygen species)and decrease in antioxidant may lead to tissue damage and different diseases[2].Antioxidant plays a major role in protecting our body by reducing the oxidative damage to cellular components caused by Free radicals[3].Phytoconstituents in plants are important source of antioxidants & are capable to terminate free radical chain reaction[4].

Curculigo orchioides (kali musli) is a tuberous,perennial herb with short or elongate root stock[5].

Root stocks are sweet,cooling, diuretic, bitter, aphrodisiac, appetizer, carminative &antipyretic[6].

2. MATERIALS&METHODS

2.1 Plant material

The roots of golden eye grass were collected from village Gumpanapalli which is about 20kms from Rampachodavarm.Theplant was identified and authenticated by T.Raghuram,Taxonomist,MaharaniCollege,Peddapuram,EastGodavari Dist, A.P

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2.2 Preparation of Extract

The freshly collected roots of plant were cleaned from dirt,dried under shade and then coarsely powdered manually.The powder was macerated in ethanol for a period of 7 days and then subjected to hot Percolation for 8hrs.Then the solution was filtered, concentrated and dried.

2.3 Chemicals and Instrument

All the chemicals used were of analyitcalgrade.DPPH was purchased from Research lab fine chem industries,Mumbai.Gallic acid is a gifted sample.Trichloroaceticacid, Phosphatebuffer, Ammonium molybdate, Sodiumphosphate, Sulphuric acid were of analyticalgrade.

The instruments used UV-Visible spectrophotometer (ELICO-SL210),centrifuge machine, electronic balance.

2.4 Invitro antioxidant study

The antioxidant activity of ethanolic root extract ofCurculigo *orchioides* was determined by three methods.All the assays are carried out in triplicate and average values are considered.

2.4 a) DPPH (2,2-diphenyl 1-picryl hydrazyl) Free Radical Scavenging Activity[7]

The free radical scavenging activity was followed by DPPH method.0.1mM solution of DPPH in methanol was prepared.Gallic acid was taken as reference standard.Different concentrations of extract (50.0,100.0,300.0, 500.0µg/ml) and standard (1.0,2.5,5.0 µg/ml) were prepared using methanol.1.0 mL of 0.1mM DPPH solution was added to 3.0 mL of all concentrations of extract and standard seperately.0.1mM DPPH and methanol were used as blank.These mixtures were kept in dark for about 30min and the absorbances were measured at 517nm.

Finally the %inhibition was calculated by using the formula

DPPH Scavenged: $\{(A_0-A_1)/A_1\}*100$

Where A_0 is the absorbance of the blank (containing all reagents except the sample extract), and A_1 is the absorbance of sample extract.

The anti oxidant activity ofethanolic root extract of *Curculigo orchioides* was expressed as IC_{50} .

The IC_{50} value is defined as concentration in (ig/ml) of extract that scavenges DPPH radical by 50%.

2.4 b) Reducing Power Assay[8]

Different concentrations of extract (50.0, 100.0, 300.0, 500.0µg/ml) and standard Gallic acid(1.0,2.5,5.0 µg/ml) were prepared using distilled water. 1.0% Potassium ferricyanide,10.0%Trichloroaceticacid, 0.1%Ferricchloride,0.2M Phosphate buffer (P^H6.6) were prepared using distilled water.Then 1.0 mL of each concentration of standard & extract were taken separately and to this 1.0 mL of phosphate buffer,1.0mL of potassium ferricyanide were added.Then these samples were incubated at 50^ocfor 20min.Then 2.5mL of 10.0%Trichloroacetic acid was added,which was then centrifuged at 3000rpm for 10min.Then upper layer (2.5mL) was separated and then add 2.5mL of distilledwater,0.5mL of freshly prepared ferric chloride was added and then absorbances were measured at 700nm.

2.4 c)PhosphoMolybdenum Assay

Different concentrations of extract (50.0,100.0,300.0, 500.0µg/ml) and standard Gallic acid(1.0,2.5,5.0 µg/ml) were prepared using distilled water. 0.3 mL of each concentration of extract and standard were combined with 3.0mL of reagent(0.6 M sulfuric acid, 28mMsodiumphosphate and 4 mM ammonium molybdate).The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using spectrophotometer against blank after cooling to room temperature.Methanol (0.3 ml) in the place of extract was used as the blank.

3. RESULTS AND DISCUSSION

DPPH Free Radical Scavenging Assay

DPPH radical scavenging assay is rapid and sensitive method for the antioxidant screening of plant extracts[10].The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517nm which is induced by antioxidants.

Table 1 shows the percentage of DPPH radical scavenged by ethanolic root extract of *Curculigo orchioides* and the standard Gallic acid.**Figure 1a,1b**illustrates a decrease in the concentration of DPPH radicals due to the soluble constituents ethanolic root extract of *Curculigo orchioides* and the standard Gallicacid as a reference compound were observed.The IC_{50} values were found to be 153.0µg/mL &3.03µg/mL for ethanolic root extract of *Curculigo orchioides* and the Gallic acid respectively.

Reducing Power Assay

Reducing power assay is based on the principle that substances which have reduction potential ,react with Potassium Ferricyanide(Fe^{+3}) to form potassium ferrocyanide (Fe^{+2}) which reacts with Ferric chloride to form Ferric Ferrous complex that has an absorption maximum at 700nm.The reducing capacity of the compound may serve as significant indicator of its potential antioxidant activity[11].

Table 2 indicates the reducing power of ethanolic root extract of *Curculigo orchioides* and the Gallic acid.From **Figure 2a,2b** it was found that the absorbance of ethanolic root extract of *Curculigo orchioides* increased with increase in concentration.

Phosphomolybdenum Assay

Phosphomolybdenum Assay is based on the reduction of MO (VI) - MO(V) and forms green colouredphospho molybdenum(V) green complex at acid P^Hwhich shows maximum absorbance At 695nm.

Table 3 indicates the antioxidant capacity of ethanolic root extract of *Curculigo orchioides* and the Gallic acid.From **Figure 3a, 3b** it was found that the absorbance of ethanolic root extract of *Curculigo orchioides* increased with increase in concentration.

Table 1: DPPH Radical Scavenging Activity of Ethanolic Root Extract of *Curculigo orchioides*

Tested Material	Concentration (µg/ml)	%Inhibition±SEM	IC_{50}(µg/ml)
Sample Extract	50.0	34.16±0.718	153.0
	100.0	37.17±0.349	
	300.0	60.06±0.042	
	500 .0	74.56±0.435	
Gallic Acid	1.0	24.37±0.109	3.03
	2.5	40.67±0.045	
	5.0	66.14±0.418	

Values are expressed as mean±SEM, n=3

Table 2: Reducing Power of Ethanolic Root Extract of *Curculigo orchioides*

Tested Material	Concentration (µg/mL)	Absorbance±SEM
Sample Extract	50.0	0.0871±0.007
	100.0	0.3014±0.003
	300.0	0.7476±0.004
	500 .0	0.9000±0.003
Gallic Acid	1.0	0.0088±0.002
	2.5	0.0331±0.003
	5.0	0.0708±0.005

Values are expressed as mean±SEM, n=3

Table 3: Antioxidant Capacity of Ethanolic Root Extract of *curculigo orchioides*

Tested Material	Concentration(µg/mL)	Absorbance±SEM
SampleExtract	50.0	0.0066±0.001
	100.0	0.0465±0.004
	300.0	0.1709±0.004
	500 .0	0.3153±0.007
Gallic Acid	1.0	0.0391±0.003
	2.5	0.1284±0.002
	5.0	0.2001±0.001

Values are expressed as mean±SEM, n=3

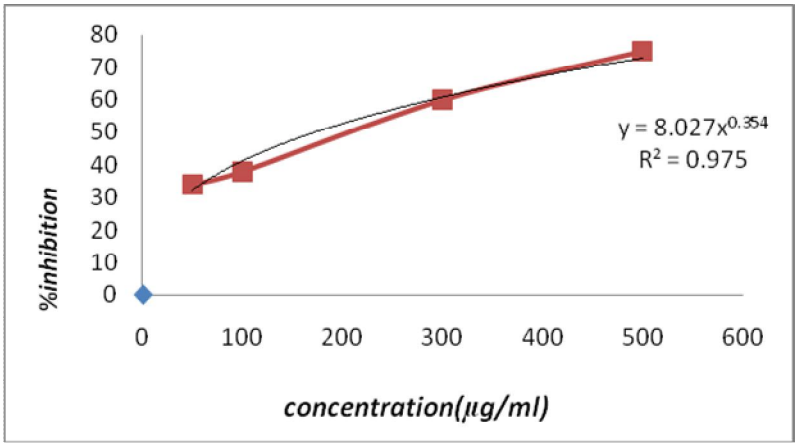

Fig. 1a: Free radical scavenging activity of Ethanolic Extract of *Curculigo orchioides*

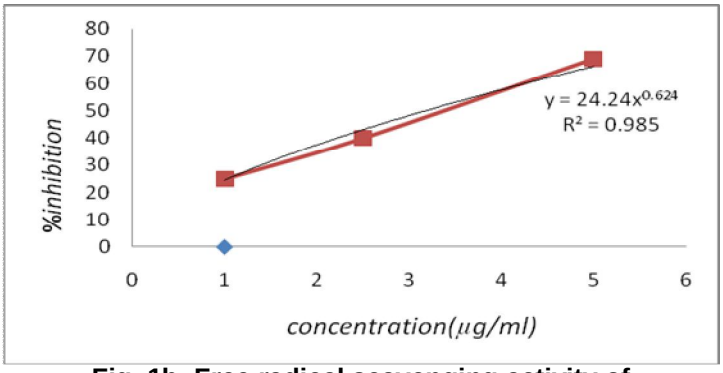

Fig. 1b: Free radical scavenging activity of Reference Standard Gallic Acid

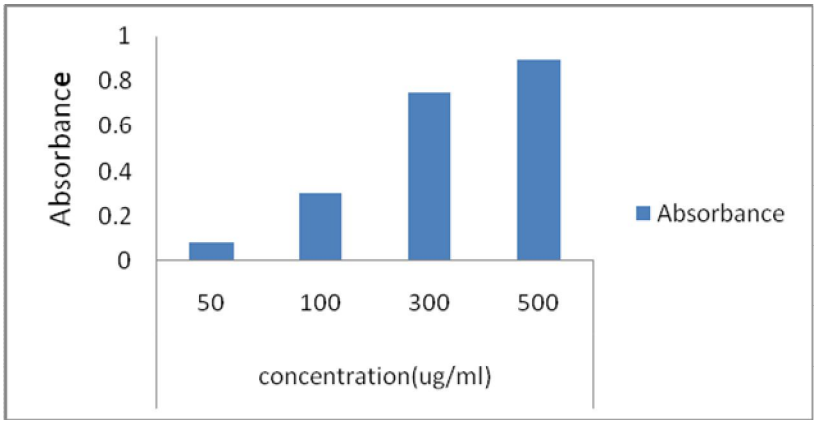

Fig. 2a: Reducing Power of Ethanolic Extract of *Curculigo orchioides*

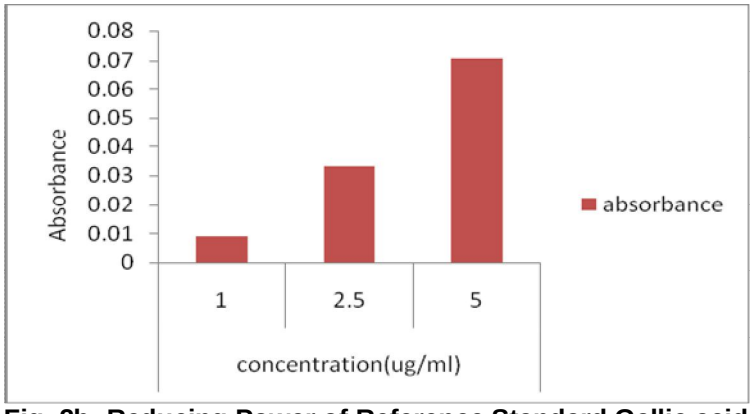

Fig. 2b: Reducing Power of Reference Standard Gallic acid

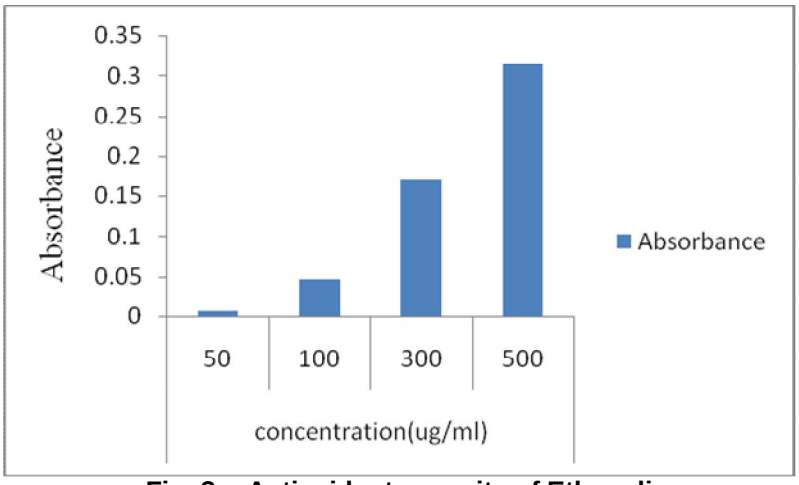

Fig. 3a: Antioxidant capacity of Ethanolic root extract of *Curculigo orchioides*

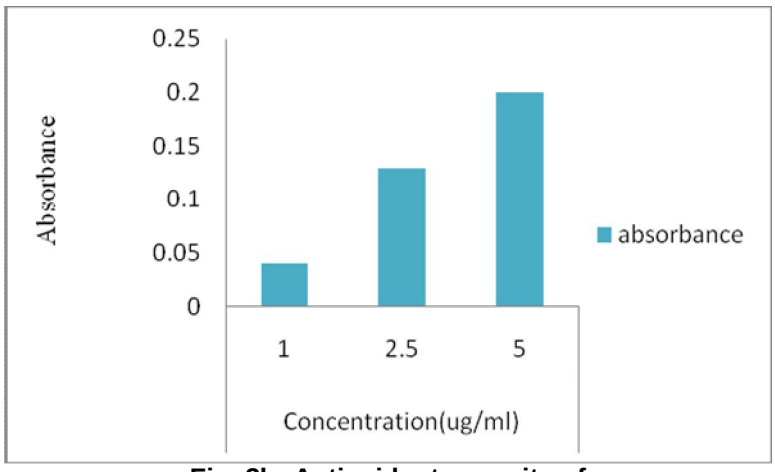

Fig. 3b: Antioxidant capacity of Reference Standard Gallic Acid

CONCLUSION

The study was performed to evaluate the invitro antioxidant activity of ethanolic root extract of *Curculigo orchioides.* The results obtained indicates the significant antioxidant activity in all the three methods and the results were compared with standard reference drug Gallic acid.Further research investigations may be carried out to isolate the actual phytoconstituents responsible for Antioxidant activity.

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