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RESEARCH ARTICLE

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IN-VITRO ANTIOXIDANT ACTIVITIES OF METHANOLIC EXTRACT OF ROOT OF *coleous vettiveroides (*jacob*)*

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ABSTRACT

In vitro antioxidant activities of methanolic extract of tuberous root of *coleous vettiveroides* (jacob.) was investigated. The free radical scavenging activity to evaluate by DPPH (2,2 -diphenyl -1- picryl hydrazyl) method, Nitric oxide method, Total antioxidant activity. DPPH radical scavenging activity of Methanolic extract and reference standard Rutin IC_{50} values was found to be 250 μg/ml and 800 μg/ml, nitric oxide scavenging activity of methanolic extract and reference standard ascorpate IC_{50} values was found to be 510 μg/ml and 490 μg/ml and Total antioxidant activity of methanolic extract was evaluated by phosphomolybdic acid method and reference standard ascorbate IC_{50} values was found to be 560μg/ml and 585 μg/ml respectively. The above result of possess good antioxidant activity when compared to the above all standards.

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KEYWORDS

Antioxidant, *coleous vettiveroides*, DPPH method, Nitric oxide method, total antioxidant activity

INTRODUCTION

Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's disease, mongolism, ageing process and perhaps dementia (Polterat 1997).Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical-induced tissue injury(Pourmorad et al*.,* 2006) Antioxidants thus play an important role of protecting the human body against damage by reactive oxygen species (Lollinger J et al.,1981,TutourBL et al.,1990). The root of *coleous vettiveroides* (Jacob) belongs to the Lamiaceae family**.** It is a small and succulent herb, with pubescent leaves and fibrous roots, which are strongly aromatic. This plant exhibits anti-bacterial, antipyretic and analgesic activity (Meyerhoff, 1978a). It is also used for the emmenagogue in India (Yoganarasimhan, 2000).

MATERIAL AND METHODS

Collection and Identification of Plant materials

The whole plant of *coleous vettiveroides* (Jacob), were collected from Sirkali, Nagapatinam District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants, Siddha Unit, Government of India. Palayamkottai. The whole plant of *coleous vettiveroides* (Jacob)*,* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powdered materials were successively extracted with methanol by hot continuous percolation method in Soxhlet apparatus for 24 hrs (Harborne J.B.1984). The extract was concentrated by using a rota evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Evaluation of Antioxidant activity by in vitro Techniques

1. DPPH photometric assay (Mensor,L.L et al.,2001)

In this method ____was assayed by methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavengingactivity(\%) = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\ \text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical+ methanol; A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

2. Nitric oxide method (DC.Garrat et al., 1964)

Nitric oxide generated from sodium

nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat. The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25^0C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted out and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological P^H spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

3. Total antioxidant activity (Phosphomolybdic acid *method) (P.M.Prieto et al)*

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (Prieto et al., 1999). An aliquot of 0.4 ml of sample solution was combined in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28milimole sodium phosphate and 4 milimole ammonium molybdate). These vials were capped and incubated in a water bath at 95ºc for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expressed relative to that of ascorbic acid.

RESULT AND DISCUSSION

I. DPPH METHOD

The percentage of DPPH radical scavenging activity of methanolic extract of *coleous vettiveroides is* presented in Table 1. The methanolic extract of *coleous vettiveroides* exhibited a maximum DPPH scavenging activity of 55.08% at 1000 µg/ml whereas for Rutin (standard) it was found to be 61.75% at 1000 µg/ml. The IC_{50} of the methanol extract of *coleous vettiveroides* and Rutin were found to be 250 µg/ml and 800µg/ml respectively

Table 1
Effect of Methanolic extract of coleous vettiveroides (Jacob) on DPPH assay

S.No	Concentration (µg/ml)	% of activity (±SEM*)	
		Sample (Methanolic extract)	Standard (Rutin)
1	125	29.06±0.032	15.80 ± 0.056
2	250	45.02±0.045	29.08 ± 0.043
3	500	51.02±0.011	45.21 ± 0.028
4	1000	55.08±0.012	61.75 ± 0.011
		IC_{50} = 250µg/ml	**IC_{50} = 800µg/ml**

All values are expressed as mean ± SEM for three determinations

2. NITRIC OXIDE METHOD

Free radical scavenging activity of the methanolic extract of *coleous vettiveroides* was determined by nitric oxide method. The free radical scavenging potential shown maximum

activity is 55.45% at 1000µg/ml for as Standard (ascorbate) was found to be 67% at 1000 µg/ml. The IC_{50} of the methanolic extract of *coleous vettiveroides* and standard (ascorbate) was found to be 510µg/ml and 490µg/ml better antioxidant is respectively

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Table-2.
Antioxidant activity of root of Methanolic extract of coleous vettiveroides by nitric oxide free radical scavenging method

S.No	Concentration (µg/ml)	% of activity (±SEM)	
		Sample (Methanolic extract)	Standard (ascorbate)
1	125	30.61±0.02	28.43±0.045
2	250	45.24±0.19	47.45 ±0.034
3	500	49.14±0.01	57.16±0.032
4	1000	55.45±0.05	67.07±0.053
		IC_{50}= 510µg/ml	IC_{50}=490µg/ml

****All values are expressed as mean ± SEM for three determinations***

3. TOTAL ANTIOXIDANT METHOD

Total antioxidant activity of the methanolic extract of *coleous vettiveroides* was determined by phosphomolybdate method. The free radical scavenging potential shown

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maximum activity is 72% at 1000µg/ml; for as Standard (ascorbate) it was found to be 69% at 1000 µg/ml. The IC_{50} of the methanolic extract of *coleous vettiveroides* and standard (ascorbate) was found to be 560µg/ml and 585µg/ml better antioxidant respectively.

Table-3.
Antioxidant activity of root of Methanolic extract of coleous vettiveroides (Jacob) by Phosphomolybdate method

S.No	Concentration (µg/ml)	% of activity (±SEM)	
		Sample (Methanolic extract)	Standard (ascorbate)
1	125	45.22±0.02	31.53±0.051
2	250	55.31±0.01	45.51 ±0.014

3	500	61.43±0.08	59.32±0.042
4	1000	69.30±0.87	72.00±0.024
		IC_{50}= 560 μg/ml	IC_{50}=585μg/ml

All values are expressed as mean ± SEM for three determinations

CONCLUSION

The results of the above investigation indicated that the methanolic extract of root of *coleous vettiveroides* showed strong antioxidant activity. However, Phytochemical screening of methanolic extract showed presence of Triterpenoids, Phenolic compound, proteins and Flavonoids,carbohydrates. So it can be concluded that these components might be involved in the antioxidant activity of *coleous vettiveroides*

REFERENCE

1. Polterait O. (1997) Anti Oxidants and free-radicalScavengers of Natural origin Current Org. Chem. 1.415-440
2. Pourmorad F, Hosseinimehr SJ, Shahabimajd N (2006). Antioxidantactivity, phenols, flavanoid contents of selected Iranian medicinal plants. S. Afr. J. Biotechnol. 5: 1142-1145.
3. Lollinger J: Free radicals and food additives. Taylor and Francis London; 1981:21.
4. Tutour BL: Antioxidative activities of algal extracts. *Synergistic effect with vitamin E. Phytochemistry* 1990, 29:3759-3765
5. Harborne J.B. (1984) Phytochemical methods 11 Edn. In Chapman &, Hall. New York: 4-5.
6. Mensor , L.L, Meneze, F.S., Leitao, G.G., Reis, A.S., Dos santor, J.C., Coube, C.S and Leitao,S.G (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother.Res.15, 127-130.
7. DC.Garrat, The quantitative analysis of drugs, Champman and Hall, Japan, 3, 456-458 (1964).
8. P.M.Prieto,M.Aguilar, Anal. Biochem, 269,337-341(1999). Yoganarasimhan, 2000 S.N. Yoganarasimhan, Medicinal Plants of India, Tamil Nadu. V. Srinivasan, N. Kosal Ram, Cyber Media, Bangalore (2000) p. 2
9. Meyerhoff, 1978a. *P. tetensis* (Bak.) Agnew. Herbarium specimen collected from Kenya (*Meyerhoff)*14M.