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PHYTOCHEMICAL SCREENING AND IN-VITRO ANTI-OXIDANT ACTIVITY OF METHANOLIC LEAF EXTRACT OF *COLDENIA PROCUMBENS* LINN.

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ABSTRACT

Anti-oxidant plays a major role in the prevention of carcinogenesis. In the present study we tried evaluating the anti oxidant activity of methanolic extract of Coldenia procumbense leave. The phytochemical screening indicated the presence of steroids, flavonoids, Alkaloids, Phenols, Glycosides in the methanolic extract. Antioxidant value of the extract was evaluated by using Hydrogen peroxide radical scavenging assay and reducing power method. In both the methods Ascorbic acid is used as standard and it was observed that Coldenia procumbense showed considerable inhibition (84 - 95%) when compared with that of standard. Reducing power of extract was increased with concentration thus showing that the antioxidant potential is dose dependent. Thus proving that the methanolic extract of Coldenia procumbens leaves is capable of antioxidant activity even though further detailed molecular level study is in need.

KEYWORDS: Coldenia procumbens, Phytochemical Screening, Antioxidant, Hydrogen peroxide, Reducing Power Method.

INTRODUCTION

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. The chemical constituents present in plants are a part of the physiological functions of living flora, hence are believed to have better compatibility with the human body.[1] Oxidative stress (OS) is a phenomenon associated with the action of free radicals and reactive metabolites in the organism. Free radicals participate

in a large number of subsequent reactions. They are derived from basic radical molecules, such as superoxide anion radical, shortly superoxide O_2, or nitric oxide. Newly formed metabolites have a great oxidative ability and they are often more reactive thantheir maternalmolecules such as the most reactive hydroxyl radical, HO or non-radical molecules such as hydrogen peroxide, singlet oxygen, and peroxynitrite or hypochlorous acid.[2] Polyphenols of plant raw materials are mostly significant for their antioxidant properties. The antioxidant activity of polyphenols is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators and reductant of ferryl haemoglobin.[4,5]

MATERIALS AND METHODS

PLANT COLLECTION AND EXTRACTION

Coldenia procumbens plants are collected manually from the loamy soil of Medak district, Telangana. They are washed, leaves are separated and shade dried. These dried leaves are pulverized to attain a coarse powder. *Coldenia procumbens* leaves powder is extracted by cold maceration using methanol for 7 days with proper intermediate shaking and the macerate was filtered into a container. The extract is concentrated in an evaporator. The dried residue was stored in a desiccator. This extract is used for preliminary phytochemical screening and also for *In- vitro* anti oxidant activity screening.

IN-VITRO ANTI- OXIDANT METHODS

HYDROGEN PEROXIDE RADICAL SCAVENGING

Plant extract of various concentrations (20,40,60,80 µg/ml was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution is observed at 230 nm against blank solution containing the H_2O_2 without plant extract. All the observations were performed in triplicate an average of these values is calculated and tabulated. Ascorbic acid used as a positive control with H_2O_2 solution. The percentage inhibition was measured by comparing the absorbance of control and test.[6,7]

H_2O_2 scavenging activity = $[(A_{control} - A_{test})/ A_{control}] \times 100$

Where, $A_{control}$ = Absorbance of control reaction and

A_{test}= Absorbance in the presence of the samples of extracts

REDUCING POWER METHOD

Procedure

The reducing power was determined using the method proposed by Athukorala et al. (2006). 1.0 ml extract is mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM) and incubated at 50°C for 20 min. Thereafter, 2.5 ml of trichloroacetic acid (600 mM) is added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) is mixed with 2.5 ml of distilled water and 0.5 ml of FeCl3 (6 mM) and absorbance is measured at 700 nm. Ascorbic acid was used as positive control.[8] The % inhibition was calculated using formula and a graph is plotted to compare with that of standard.[9]

Formula

% inhibition = [O.D.of control - O.D. of Test/O.D. of control] χ 100

RESULTS AND DISCUSSIOIN

Physical status of extracts

The obtained methanolic extract of Coldenia procumbens was a dark greenish semisolid. The percentage yield was found to be 57% w/w.

Preliminary Phytochemical Screening

Preliminary Phytochemical screening was performed using standard analysisprocedures and reagents'. Results are summarized in the table given below.

Table I: Result for Methanolic extract of *Coldenia procumbens* during phytochemical screening.

Phytoconstituent	Methanolic extract
Alkaloids	+
Flavonoids	+
Steroids	+
Terpenoids	+
Tannins	-
Amino acids & Proteins	+
Carbohydrates	-
Phenols	+
Glycosides	+

Note:+ indicatesPresenceand – indicates absence of phytochemical constituents in the extract.

From the above results presence of Alkaloids, Flavonoids, Steroids, Phenols and Glycosides was observed in the methanolic extract of *Coldenia procumbens.*

Antioxidant Activity of *Coldenia procumbens* Hydrogen Peroxide Radical Scavenging Assay.

Table II: Effect of *Coldenia procumbens* on inhibition of hydrogen peroxide Radical Scavenging Assay.

S. No	Concentration (µg/ml)	Percentage Inhibition (%)	
		Ascorbic acid	Methanolic extract
1	20µg/ml	74.10±0.243	70.00±0.282
2	40µg/ml	77.20±0.214	73.12±0.253
3	60µg/ml	77.30±0.218	73.85±0.256
4	80µg/ml	80.00±0.189	75.00±0.236

*** Values are expressed as Mean ± S.D, n = 3.

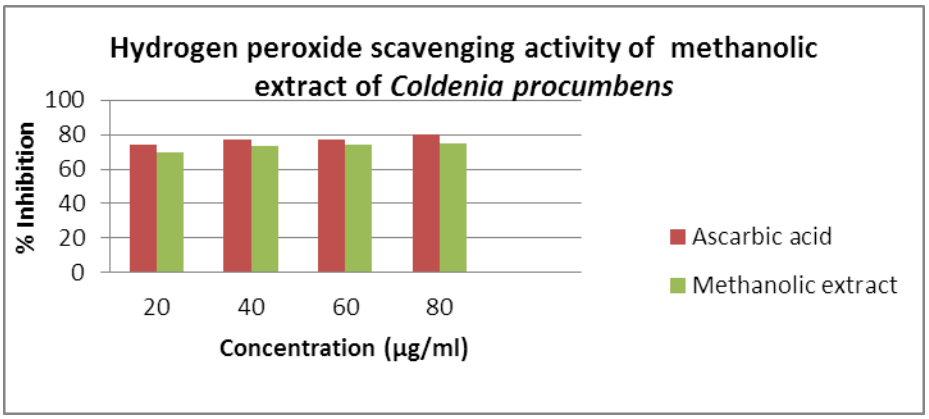

Fig.I: Hydrogen peroxide scavenging activity of methanolic extract of *Coldenia procumbens* comparison with a standard Ascorbic acid.

The effect of different concentration of *Coldenia procumbens* on inhibition of Hydrogen peroxide was given in Fig.I. The concentration dependent scavenging activity on Hydrogen peroxide was found to be significant when compared to standard Ascorbic Acid. Thus it shows that methanolic extract of *Coldenia procumbens* is capable of inhibiting the reactive oxygen species, Hydrogen peroxide.

Antioxidant Activity of *Coldenia procumbens* using Reducing Power Method.

Table III: Reducing power activity of *Coldenia procumbens.*

S.No	Concentration (µg/ml)	Reducing power activity (Absorbance at 700 nm)	
		Ascorbic acid	Methanolic extract
1	20 µg/ml	0.219 ±0.073	0.279±0.09
2	40 µg/ml	0.324±0.108	0.330±0.110
3	60 µg/ml	0.465±0.155	0.483±0.161
4	80 µg/ml	0.783±0.261	0.798±0.266

****Values are expressed as Mean ± S.D, n = 3.

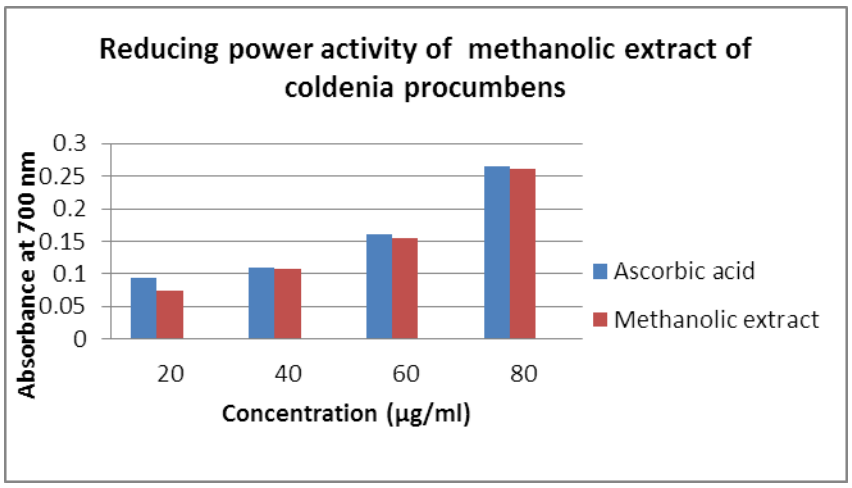

Fig. II: Reducing power activity of methanolic extract of *Coldenia procumbens* comparison with a standard Ascorbic acid.

Higher absorbance indicates greater reducing ability of the extract. As there is gradual increase in absorbance with increase in the concentration the methanolic extract of *Coldenia procumbens*, it can be said that reducing ability of methanolic extract of *Coldenia procumbens* is significant when compared to the standard Ascorbic acid.

CONCLUSION

The present study is aimed to determine the Antioxidant activity of *Coldenia procumbens.* The leaves of *Coldenia procumbens* were collected, dried and pulverised using grinder and extracted by soxhlet apparatus using polar solvent such as methanol. The percentage yield of methanolic extract was found to be 18% w/w with respect to dried material.

The extract was subjected to preliminary phytochemical screening using standard qualitative analytical methods. The extract was found to be rich in phytochemical constituents such as glycosides, flavonoids, steroids, tannins, etc.

Antioxidant property of methanolic extracts of *Coldenia procumbens* was evaluated by two methods namely, hydrogen peroxide, reducing power using ascorbic acid as standard. Reducing power of extract increased with concentration and thus proved the antioxidant potential of Methanolic extract of Coldenia procumbens. Hence, bioactive capability has been reported to be associated with antioxidant activity. They have possessed application to reduce oxidative stress with good health benefits.

The present study clearly indicated that methanolic extract of *Coldenia procumbens* possessed antioxidant activity and hence can be further presented to isolate pure phytoconstituents, to investigate and to perform other pharmacological studies.

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