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Antioxidant Activity of Methanolic Extract of *Vigna unguiculata.*

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ABSTRACT

Methanolic extract of *Vigna unguiculata* were examined for antioxidant activity by DPPH, Hydrogen peroxide scavenging activity and also reducing power assay were carried out. The antioxidant activity was found to be concentration dependent and it may be attributed to the presence of high flavanoid content in the seed of *Vigna unguiculata*. The methanolic extract has showed a very good antioxidant property compared to standard ascorbic acid. The IC-50 of the extract for DPPH and hydrogen peroxide activity was found to be 37.66±0.235µg/ml, 23±0.41 µg/ml respectively and are compared with the standard ascorbic acid which has showed 12.5±0.408 & 21.5±0.408 µg/ml in both respective methods and the absorbance of reducing power assay for the concentration of 100 µg/ml was observed to be 0.729 ± 0.0008. Hence it can be concluded that the methanolic extract of *Vigna unguiculata has* potential a very good antioxidant activity. In the present study the ascorbic acid is as reference standard and positive control. Based on our findings it can also be inferred that the methanolic extracts of *Vigna unguiculata* seeds have a potent free radical scavenging activity.

Keywords: *Vigna unguiculata*, Antioxidant activity

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INTRODUCTION

Since very old times, herbal medications have been used for relief & cure of symptoms of disease [1]. Despite the great advances observed in modern medicine in recent decades, plants are still make important contributions to health care. Much interest in medicinal plants however, emanates from their prophylactic properties, especially in developing countries. Large number of medicinal plants have been investigated for their antioxidant properties. Natural antioxidants are either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress [2].

Reactive oxygen species [ROS], sometimes called as active oxygen species, are various forms of activated oxygen molecules, which include free radicals such as superoxide ions (O2 -.) and hydroxyl radicals (OH.) as well as non-free radical species such as hydrogen peroxide (H2O2). [3] These ROS play an important role in degenerative or pathological processes, such as aging, cancers, coronary heart diseases, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammations etc [4]. Living organisms have antioxidant defence systems that protects against oxidative damage by removal or repair of damaged molecules [5]. The term 'antioxidant' refers to the activity of numerous vitamins, minerals and phytochemicals which provide protection against the damage caused by ROS [6]. Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors [7].

The natural antioxidant mechanisms maybe insufficient in variety of conditions and hence dietary intake of antioxidant compounds are important [8]. Currently the possible toxicity of synthetic antioxidants are been criticized & there is a search for a new products with antioxidative properties is a very active domain of research. Thus the interest on natural antioxidants, especially of plant origin, has given priority in recent years [9]. Antioxidants interfere with the production of free radicals & also play a key role to inactivate them [10]. Phytochemicals present in plants are strong natural antioxidants & have an important role in health care system.

Therefore the great interest has been recently focused on the natural foods, medicinal plants & phytoconstituents due to their well known abilities to scavenge free radicals [11]. Plants have antioxidative and pharmacological properties related to the presence of phenolic compounds, especially phenolic acids and flavonoids [12]. Medicinal plants are very important for the healthy lives of most of the people across the world. Several classes of biologically active compounds of these plants include alkaloids, flavonoids, tannins and phenolic compounds [13].

Phenolics are a class of secondary metabolites found in most land plants; moreover, they protect plants against ultraviolet radiation, pathogens, and herbivores. Flavonoids in biological systems are ascribed to their antioxidant abilities, capacity to transfer electrons, quenching of free radicals and chelating abilities, activate antioxidant enzymes, reduce alphatocopherol radicals and inhibit oxidases [14].

In recent years one of the area, which is attracted a great treaty of attention, is antioxidant in the control of degenerative diseases in which oxidative dent has been implicated. Various plant extracts and different lessons of phytochemicals have been shown to have antioxidant activity. Plant extracts and plant products such as flavonoids and other polyphenolic constituents have been reported to be effective radical scavengers and inhibitors of lipid peroxidation [15-19].

The aim of the present study was to carry out the antioxidant activity with the methanolic extract of *Vigna unguiculata* by using DPPH and hydrogen peroxide, reducing power assay methods. Free radicals are atoms or groups of atoms with an odd number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction. Their chief danger comes from the damage of cells, they can do when they react with important cellular components such as DNA, cell membrane etc. To prevent free radicals attack on body system if has a defense system of antioxidants. Antioxidants are intimately involved in the prevention of cellular damage which is the common pathway for cancer, ageing etc. Although there are several enzyme systems exits within the body that scavenge free radicals, the important antioxidants are vitamin E, vitamin C and beta-carotene. Most of the antioxidant compounds are derived from the plant sources [20- 22].

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MATERIALS AND METHODS

Plant material

The dry seeds of *Vigna unguiculata* were collected from local area of Gulbarga district. Preparation of crude extract: 50 grams of dry powdered seeds of *Vigna unguiculata* were & extracted successively with petroleum ether, chloroform & methanol for 10-12 hrs, through soxhlet apparatus method. The collected solution were evaporated to dryness under reduced pressure at 90^0C by rotary vaccum evaporator to obtain the respective extracts & stored in a freezer condition at -18^0C until used for further analysis. Phytochemical investigations of our previous study has showed the presence of glycosides, flavanoids & other phenolic compounds present in *Vigna unguiculata.*

Reduction of DPPH free radical

To the 1 ml of various concentrations of alcoholic and aqueous extract, 1 ml of solution of DPPH 0.1 mM (0.39 mg in 10 ml methanol) was added. An equal amount of ethanol and DPPH was added to the control. Ascorbic acid was used as the standard for comparison. After 20 minutes of incubation in the dark absorbance of colour was recorded at 517 nm [23]. Experiment was performed in triplicate. Percentage of scavenging was calculated by the following formula

$$\% \text{ Scavenging} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

Hydrogen peroxide radical scavenging activity

The ability of the extract to scavenge hydrogen peroxide was determined according to the method of Ruch, Cheng and Klaunig1989 [24]. A solution of hydrogen peroxide (2 mmol/l) (Fine Chem Industries, Mumbai) was prepared in phosphate buffer (pH 7.4). Extracts (10–100 µg /ml) were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The percentage of scavenging activity of hydrogen peroxide by MEGA was calculated using the following formula,

$$\% \text{ scavenging activity } [H2O2] = [\text{Abs (control)} - \text{Abs (standard)} / \text{Abs (control)}] \times 100.$$

Where, Abs (control): Absorbance of the control and Abs (standard): Absorbance of the extract/standard.

Reducing power assay

The reducing power of *Vigna unguiculata* extract was determined according to the method of Oyaizu 1986 [25]. Different concentration of *Vigna unguiculata* extract in 1ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$ (2.5 ml, 1%) then the mixture was incubated at 50°C for 20 min. Trichloroacetic acid (10%) slightly added (2.5 ml)to the mixture and centrifuged at 3,000 rpm for 10min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) then taken the absorbance at 700 nm. The reference standard of Ascorbic acid and the Blank solution contained Phosphate buffer were used [25].

RESULTS AND DISCUSSION

Phytochemical screening of *Vigna unguiculata* extract in our earlier studies has shown presence of saponins, flavanoids and is also a rich source of polyphenols. This indicates that the high soluble phenolics as well as flavanoids present in the *Vigna unguiculata* extract could have the strong free radical scavenging activity in all used assay. Free radicals mainly the reactive oxygen species (ROS) are involved in initiation, promotion and progression of carcinogenesis [26]. ROS induced oxidative damage of DNA and other cellular components leading to cancer related mutations [27]. Consequently antioxidants play an important role in the protection of human body against damaged caused by reactive oxygen species [28]. DPPH is a compound that

consists of a nitrogen free radical which is easily quenched by free radical scavengers such as phenolic compounds. DPPH scavenging method has been used extensively to predict antioxidant capacity because of the relatively short time required for analysis. The free radical scavenging activity of the extract increases depending on extract concentration [29].

Presence of phenolics and flavanoids in *Vigna unguiculata* might be responsible for DPPH, Hydrogen peroxide assay and reducing peroxide assay. In this present study methanolic extract of *Vigna unguiculata* was subjected to evaluate its in vitro antioxidant activity. The in vitro antioxidant potential of the *Vigna unguiculata* extract was evaluated by DPPH free radical scavenging activity, hydrogen peroxide scavenging activity and reducing power assay. In vitro antioxidant activity of the *Vigna unguiculata* was carried out in triplicate using ascorbic acid as standard. The extract of *Vigna unguiculata* posses good DPPH scavenging activity. The methanolic extract of *Vigna unguiculata* has showed the percent inhibition of DPPH as 22.6, 45.8, 61.3, 63.2 & 83.9 for the concentration of 20, 40, 60, 80, 100 µg /ml. The percent inhibition of DPPH with same concentration of ascorbic acid has showed 83.4, 89.8, 94.6 & 98.42. From this it is evident that the extract of *Vigna unguiculata* serve as free radical inhibitors or scavengers.

Phenolic compounds are known as powerful chain breaking antioxidants. Phenols are very important plant constituents because of their scavenging ability due to the presence of hydroxyl groups it may contribute directly as antioxidative agent. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans [30], similar studies have been carried out by Rajani Kanta sahu et al [31] and Kandhasamy S et al [32].

Hydrogen peroxide is a weak oxidizing agent and it is not very reactive, can cross biological membranes. Because of possible involvement of hydrogen peroxide in the generation of hydroxyl radicals, this property places hydrogen peroxides in a more prominent role to initiate cytotoxicity than its chemical reactivity. Thus removing of hydrogen peroxide is very important for the protection of living system. The extract of *Vigna unguiculata* has showed the percent inhibition of hydrogen peroxide scavenging activity as 10.7, 14.0, 17.2, 20.1, 22.9 for the concentration of 20, 40, 60, 80, 100 µg /ml respectively. The percent inhibition of hydrogen peroxide scavenging activity with same concentration of ascorbic acid has showed 43.96, 52.25, 64.50, 76.93 & 87.56. Our findings are similar to the previous studies of Patil SM et al [33] & Babu rao et al [34].

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant same. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound .presence of reducers causes the conversion of ferricyanide. Complex used in this method to ferrous form [35]

The reducing agent are having the availability of atoms which can donate electron and react with free radicals and then convert them into more stable metabolites and terminates the radical change reaction. In the present methanolic extract of *Vigna unguiculata* showed the optical absorbance of 0.669, 0.672, 0.673, 0.688, 0729, at concentration of 20,40,60,80 & 100 µg/ml. The optical absorbance of standard ascorbic acid at the same concentration was 0.873, 1.018, 1.045, 1.186, and 1.233 respectively. Similar findings have been shown by S.Jamuna et al [36], A hemalata et al [37].

On the basis of the results obtained in the present study it is observed that the methanolic extract of *Vigna unguiculata* which contains phenolic compounds that exhibit a high antioxidant and free radical scavenging activities. These in vitro assays indicate that the methanolic extract of *Vigna unguiculata* has significant source of natural antioxidant compound, which might be helpful in preventing the progress of various oxidative stress. Further study on this plant might provide the isolation of some active constituents rendering the antioxidant potential is under progress.

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Table 1: Showing % Inhibition of DPPH by different concentration of extract and ascorbic acid

Sl no	Concentration µg/ml	% Inhibition Extract Mean ±SD	% Inhibition Ascorbic acid Mean ±SD
1	20	22.6 ± 0.124	83.4 ± 0.699
2	40	45.8 ± 0.169	89.8 ± 0.498
3	60	61.3 ± 0.169	94.6 ± 0.205
4	80	63.2 ± 0.244	98.0 ± 0.249
5	100	83.9 ± 0.169	98.42 ± 0.057
6	IC50 Value µg/ml	37.66±0.235	12.5 ± 0.4

Extract and ascorbic acid shows IC 50 values 37.66±0.235 and 12.5±0.4 µg/ml respectively.

Table 2: Showing % Inhibition of H_2O_2 free radical by different concentration of extract and ascorbic acid

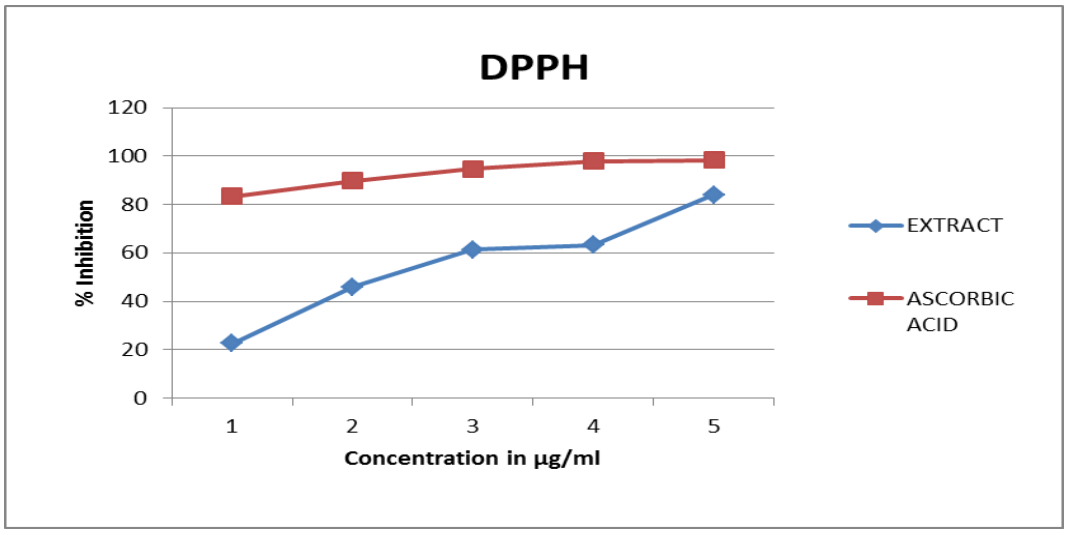
Sl no	Concentration µg/ml	% Inhibition Extract Mean ±SD	% Inhibition Ascorbic acid Mean ±SD
1	20	10.7 ± 0.124	43.9 ± 0.008
2	40	14.0 ± 0.124	52.2 ± 0.0124
3	60	17.2 ± 0.163	64.5 ± 0.0124
4	80	20.1 ± 0.169	76.9 ± 0.0124
5	100	22.9 ± 0.124	87.5 ± 0.0124
6	IC50 Value µg/ml	23.0 ± 0.41	21.5 ± 0.4

Extract and ascorbic acid shows IC 50 values 23±0.41and 21.5±0.4µg/ml respectively.

Table 3: Showing the reducing power assay of extract and ascorbic acid

Sl no	Concentration µg/ml	Reducing power of Extract Mean ±SD	Reducing power of Ascorbic acid Mean ±SD
1	20	0.669 ± 0.002	0.873 ± 0.002
2	40	0.672 ± 0.001	1.018 ± 0.0004
3	60	0.673 ± 0.0016	1.045 ± 0.002
4	80	0.688 ± 0.0004	1.186 ± 0.002
5	100	0.729 ± 0.0008	1.233 ± 0.001

Increased absorbance with increase in concentration showed the increase in reducing power of extract.



Graph 1: Showing DPPH scavenging activity of methanolic extract and ascorbic acid.

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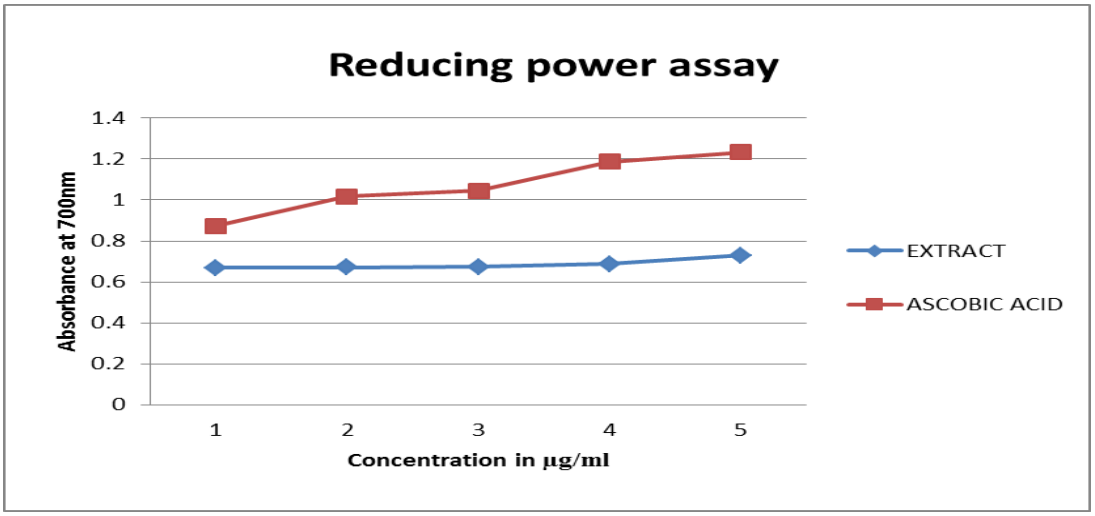
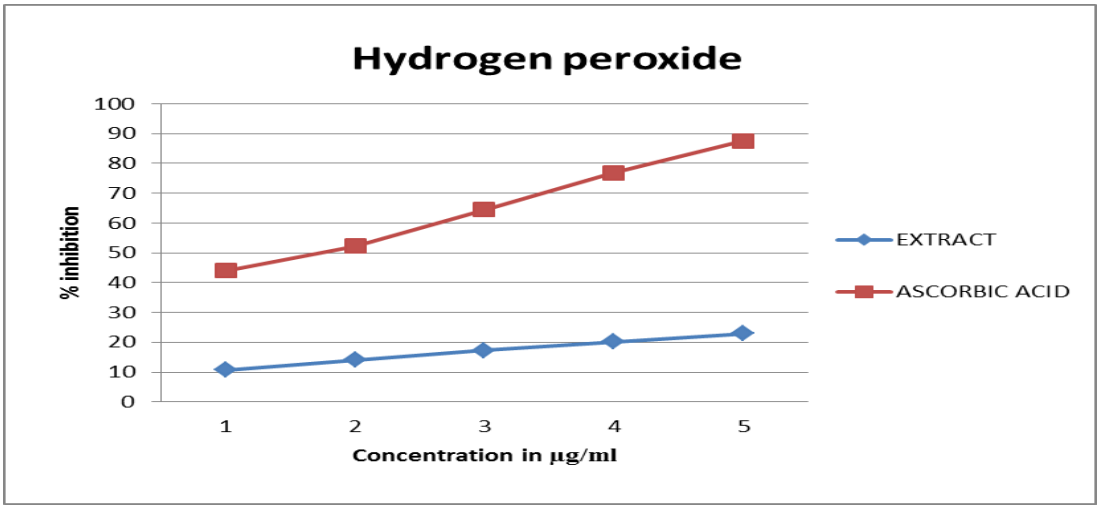


Graph 2: Showing reducing power of methanolic extract and ascorbic acid



Graph 3: Showing hydrogen peroxide scavenging activity of methanolic extract and ascorbic acid

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