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In vitro, Antioxidant effect of seed coats extracts of *Vigna mungo*

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ABSTRACT

The plant *Vigna mungo L.* is commonly known as Urid bean (Blackgram; Leguminosae family). It is an annual, semi-erect to spreading herb growing to a height of 25-90 cm. The *in-vitro* antioxidant activity of leguminous seed coat extracts of methanol and aqueous ethanol *Vigna mungo* has been investigated by 1,1-diphenyl,2-picryl–hydrazyl free radical (DPPH) and nitric oxide scavenging methods. The aqueous ethanol extract has better antioxidant activity than methanolic extract. The results have been compared with the standard ascorbic acid.

Key words: Antioxidant activity, *Vigna mungo*, Free radicals, aqueous ethanolic extract

1. INTRODUCTION

Oxidation is one of the most important free radical-producing processes in food, chemicals and even in living systems. Free radicals play an important role in food and chemical material degradation, contributing also to more than one hundred disorders in humans[1, 6]. Highly reactive free radicals and oxygen species present in biological systems can oxidize nucleic acids, proteins and lipids, initiating degenerative diseases[7,8]. Antioxidants significantly delay or prevent the oxidation of easily oxidable substrates. Plants contain high concentrations of numerous redox-active antioxidants, such as polyphenols, carotenoids, tocopherols, glutathione, ascorbic acid and enzymes with antioxidant activity, which fight against hazardous oxidative damage of plant cell components. Plant-sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytates and phytoestrogenes have been recognized as having the potential to reduce disease risk. The intake of food rich in *a*-tocopherols, *ß*-carotene and ascorbic acid has been associated with reduced oxidative-stress related diseases. Phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl, thus inhibiting the oxidative mechanism that lead to degenerative diseases[9, 12].

Legumes are the second source of proteins, carbohydrates, vitamins and minerals after corn. Legumes produce primary and secondary metabolites and other phytochemicals such as pharmaceuticals, pesticides and industrial products[13]. They are also an excellent source of nuetraceutical constituents such as fibre, protease inhibitors, phytic acid and polyphenols such as flavonoids, isoflavones, lignans and tannins. Black gram (*Vigna mungo L.*) is an important pulse crop occupying unique position in Indian agriculture. Among the pulses, it stands fourth in production and acreage[14]. It is very nutritious and is recommended for diabetics, as are other pulses, also aphrodisiac, lactagogue and nervine tonic. The seed coat colour is attributed to the presence and quantity of polyphenols such as flavonol glycosides, condensed tannins and anthocyanins. These compounds have antioxidant, antimutagenic and anticarcinogenic activities and also free radical scavenging properties .Moreover, literature on health beneficial effects of Indian plant food is scanty. However, there are no references any type of physiological activity of *Vigna mungo* seed coats. It was, therefore aimed to investigate its antioxidant activity by various *in vitro* models.

2. EXPERIMENTAL

2.1. MATERIALS AND METHODS

The seed coats of leguminous plants *Vigna mungo* were collected from Multai (Madhya Pradesh) India and authenticated by Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University. The seed coats of *Vigna mungo L.* were separated by mechanical means using hand grinder. The powdered seed coats were extracted with methanol and aqueous ethanol in a Soxhlet extractor at a temperature of 55-60 ºC, for a period of 7-8 hr. The extracts were filtered in order to obtain particle free extracts and evaporated using a rotary evaporator under vacuum at 50ºC and the extract thus obtained was used directly for the assessment of antioxidant activity through *in vitro* method.

DPPH free radical scavenging and nitric oxide scavenging activity methods are the most popular methods for the determination of antioxidant activity. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diagnostic molecule. The reduction capacity of DPPH radical was determined by the decrease in its absorbance at 517nm, which is induced by antioxidant. In nitric oxide method, the nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH, is inhibited by antioxidants, which compete with oxygen to react with nitric oxide, the absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with N-(1-naphyl) ethylenediamine (NEDA) was read at 546nm[15].

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2.2. DPPH radical scavenging activity

The free radicals scavenging activity[16] of the aqueous ethanol and methanol extract seed coats *Vigna mungo* and L- ascorbic acid (Vitamin C) measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. About 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution of compounds in DMSO at different concentrations (10-100µl/ml). Thirty minutes later, the absorbance was measured at λ_{max}=517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The Capability to scavenge the DPPH radical was calculated by using following equation:

$$\text{DPPH Scavenging Effect (\%)} = \{(A_{control} - A_{sample})/ (A_{control})\} \text{ X } 100$$

Where,
$A_{control}$ = Absorbance of the control reaction and
A_{sample} = Absorbance in the presence of the extracts.

The antioxidant activity of extract is expressed as IC50. The IC50 value is the measure of concentration in (µg/ml) of extract that inhibits 50% of DPPH radicals. The results of antioxidant activity of seed coat extract of using DPPH free radical scavenging methods are shown in Fig.1.

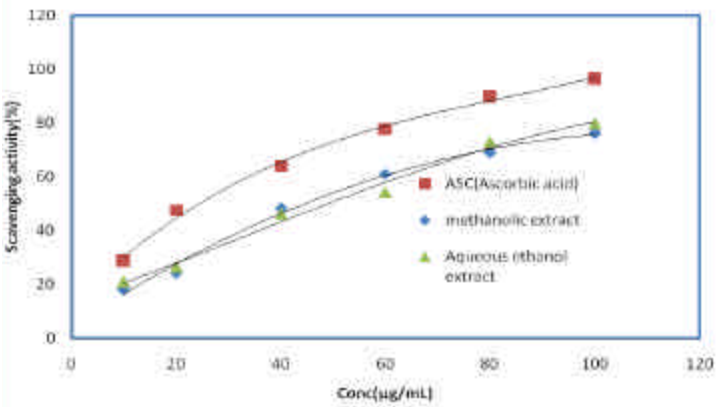

Fig 1: DPPH radical scavenging activity of extracts

2.3. Nitric oxide (NO) radial scavenging activity

The seed coat extract of *Vigna mungo* was screened for nitric oxide (NO) radical scavenging activity. 1 ml sodium nitropruside (10 mM) in 0.5 M phosphate buffer (pH 7.4) was mixed with 3.0 ml of the different concentrations (10- 100 µg/ml) of the sample dissolved in methanol and incubated at 25 ^{0}C for 15 min. Above samples were reacted with Greiss reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% N-(1-napthyl) ethylenediamine dihydrochloride in water). The absorbance of the chromophore formed during the diazotization of nitrate with sulphanilamide and subsequent coupling with N- (1-napthyl) ethylenediamine was read at λ_{max}=546 nm. The same reaction mixture without extract of plant but with equivalent amount of 0.5 M phosphate buffer served as control. Ascorbic acid was used as positive control (Fig .2).

The capability to scavenge the NO radical was calculated using the following equation:

NO Scavenging Effect (%) = {($A_{control} - A_{sample}$)/ ($A_{control}$)} X 100

Where,
$A_{control}$ = Absorbance of the control reaction and
A_{sample} = Absorbance in the presence of the extracts.

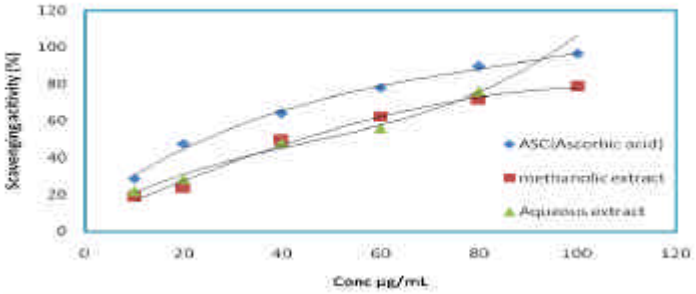

Fig 2: Nitric oxide radical scavenging activity of extracts

3.0. RESULT AND DISCUSSION

In DPPH test the ability of a compound to act as donor for hydrogen atom or electron was measured spectrophotometrically. In nitric oxide scavenging activity, the sodium nitropruside solution spontaneously generates nitric oxide which reacts with oxygen to produce nitric ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduce production of nitric ions. It is observed that aqueous ethanol seed coats extract of *Vigna mungo* shows better antioxidant activity than methanol extracts seed coats and phenolic compounds are responsible for antioxidant activity[17, 18].

4.0. CONCLUSION

The above data indicates results that *Vigna mungo* seed coat of aqueous ethanolic extract possesses significant antioxidant activity.

5.0. ACKNOWLEDGEMENTS

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