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In vitro antioxidant activity of seed extracts of *Benincasa hispida*

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ABSTRACT

In vitro antioxidant activity of methanolic and petroleum ether extract of seeds of Benincasa hispida was determined by DPPH free radical scavenging assay, Assay of reducing power method. α-tocopherol (vitamin-E) was used as standard and positive control for all analysis. All analysis was made with the use of UV-Visible spectrophotometer. The methanolic extract and petroleum ether extract of seeds of Benincasa hispida had shown very significant DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging activity compared to standard antioxidant. The DPPH radical scavenging activities of the extracts were increased with the increasing concentration. In DPPH free radical scavenging assay IC_{50} value of methanolic extract and petroleum ether extract of seeds of Benincasa hispida were 5.167µg/ml, 6.157µg/ml µg/mL whereas, IC_{50} value of α-tocopherol was 4.15µg/ml. The results concluded that the extracts have a potential source of antioxidants of natural origin.

Key words: Antioxidant, *Benincas hispida*, free radical, DPPH.

INTRODUCTION

Free radicals are the molecules that have unpaired electron in outer shell and most of which include reactive oxygen species (ROS) which have been implicated as a causative factor in the etiology of cancer and several other degenerative diseases, including Alzheimer and Parkinson [8]. Reactive oxygen species (ROS) *in vivo* include not only oxygen centered radicals such as such superoxide and hydroxyl radicals but also some non-radicals derivative of oxygen, hydrogen peroxide, singlet oxygen and hypochlorous acid. ROS in the form of superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$) are natural by-products of our body's metabolism [7]. Oxidative stress is the state of imbalance between the level of antioxidant defense system and production of ROS [15]. High oxidative stress may contribute to the development of cardiovascular disease, neurodegenerative disorders and even cancers. Solution to this is natural antioxidant (polyphenols and flavonoids) that has the ability to scavenge the free radicals and thus substantially prevent damage to biomolecules. Although the mammalian body has certain defense mechanisms to combat and reduce oxidative damage, epidemiological evidence indicates that the consumption of foodstuffs containing antioxidant phytonutrients notably flavonoids and other polyphenolics is advantageous for our health [4]. Natural antioxidants are essential for good health. Even though some synthetic antioxidant likes BHA, BHT, α-tocopherol and EDTA are in market but due to their toxic and harmful effects people are inclining towards safe and reliable source of plant natural antioxidant [20]. Antioxidants delay or prevent the oxidation of a given substrate by free radicals Antioxidants are an ancillary defense against oxidative stress. For example, flavonoids and polyphenols prevent hydroxyl radical-induced damage. Hydroxyl radicals may accumulate because there is no cellular enzyme to neutralize the species. They are generated by Fenton chemistry via the reaction of H_2O_2 and iron. Flavonoids inhibit hydroxyl radical production by chelating the transition metal. They

also scavenge hydroxyl radicals by donating an electron to neutralize the species in addition; some flavonoids may associate with the oxidizable substrate to prevent direct hydroxyl radical damage [1]. Lipid peroxidation and protein/DNA oxidation studies document these activities. However, most activity studies focus on the relationship between flavonoid structure and antioxidant mechanism [6]. A potent scavenger of these free radical species may serve as a possible preventive intervention for free radical mediated diseases. Recent studies showed that a number a plant products including polyphenolic substances (e.g., flavonoids and tannins) and various plant or herb extracts exert potent antioxidant actions [11, and 13]. Studies on local plants such as turmeric (*Curcuma domestica*), betel leaf (*Piper betel*), pandan leaf (*Panadanus odorus*), asam gelugur (*Garnicia atroviridis*), menkudu (*Morinda citrifolia*), pegaga (*Centella asiatica*), ginger (*Zingiber officinate*), cassava shhot (*Manihot asculenta*), *Lippia Alba*, kesum (*Polygonam minus*), *Limonia crenulata* and selom (*Oenathe javanica*) [10, 12, 16, 18, 21 and 23] exhibits good antioxidant activity.

MATERIALS AND METHODS

Plant materials: The fresh fruit of *Benincasa hispida* was collected from Agra city of Uttar Pradesh in January 2009. Seeds were separated from fruit and oven dried at 40˚C for 48 hours.

Preparation of extract: 100g dry seeds were grinded with acetone and dry overnight at room temperature. 50g seed powder suspended in methanol and 50g in petroleum ether (60-80) and keep for 6 days on shaker at 37˚C. Filtered the extract through muslin cloth (warp 22 ± 1 and 18 ± 1 per centimeter), collected filtrate keep in oven at 40˚C till methanol and petroleum ether total evaporate. Methanol extract was dissolved in methanol and petroleum ether extract was dissolved in DMSO and kept on 2-5˚C according to [17, 19].

Antioxidant assay: The antioxidant activity of plant seed extracts were determined by different *in vitro* methods such as, DPPH free radical scavenging assay, assay of reducing power methods. All assays were carried out in triplicate and average value was considered.

DPPH Radical Scavenging Activity:
Free radical scavenging activity was also performed by DPPH (2, 2'-diphenyl-1-picrylhydrazyl) free radical scavenging assay according to [3]. In this assay, the DPPH reagent was prepared by dissolving the 0.04g DPPH to 95% of 100ml methanol. Then the 2.95ml of DPPH reagent add to the 0.05ml extract with varied concentration (5, 10, 15, 20, 25, 30, 35, 40 microgram per ml). Then the absorbance of reaction mixture was measured at 517nm using spectrophotometer. α-tocopherol (1mg per ml) used as standard and the DPPH without protein sample used as control. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenging effect \%} = (A_0\text{-}A_1/A_0)\ x100$$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of plant sample and standard compounds.

Assay of reducing power:
The total reducing power of plant protein was determined by potassium ferricyanide [K3Fe(CN6)] according to [8]. In this assay 1ml extract was with varied concentration (5, 10, 15, 20, 25, 30, 35, 40 microgram per ml) was mixed with 2.5ml of 0.2M potassium phosphate buffer (pH 6.6) and 2.5ml potassium ferricyanide [K3Fe(CN6)] (10g/l), then the mixture was incubated at 50˚C for 20 minutes. 2.5ml of trichloroacetic acid (100g/l) was added to the mixture. Then the mixture was centrifuged at 3000rpm for 10 min. then the supernatant was collected and 0.5ml ferric chloride (1g/l) and 2.5ml distilled water was mixed with 2.5ml of supernatant keep for 10 minutes at normal temperature. After 10 minutes the absorbance measured at 700nm in UV visible spectrophotometer.

α- tocopherol (1mg/ml) used as standard and potassium phosphate buffer used as blank solution. The experiment was done in triplicates.

RESULTS AND DISCUSSION

In this present study the antioxidant activity of the methanolic and petroleum ether extracts of seeds *Benincasa hispida* were investigated by using DPPH scavenging assay, assay of reducing power methods. All methods have proven the effectiveness of the methanolic and petroleum ether extract compared to the standard antioxidant α-tocopherol (Vitamin E). The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of colour.

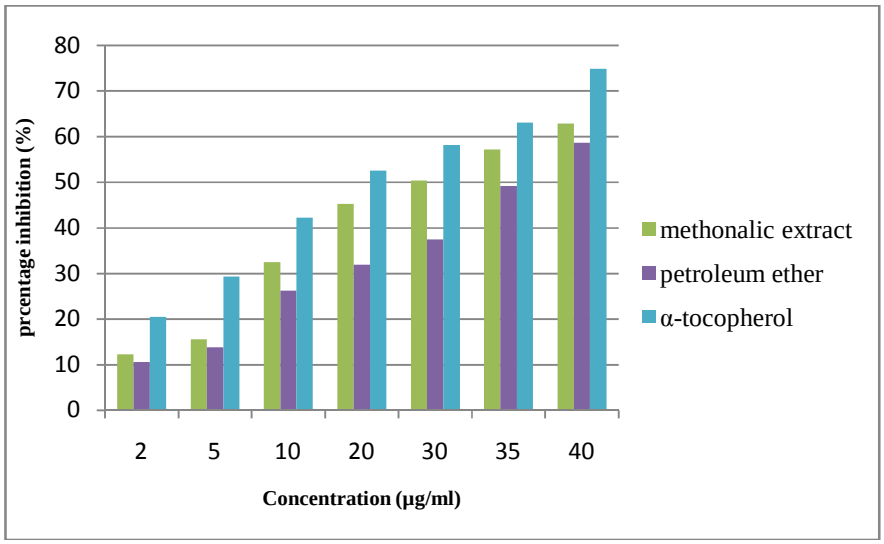

Figure 1: DPPH radical scavenging activity of methanolic extract and petroleum ether extract of seeds of *Benincasa hispida* added to solution of DPPH and radical scavenging activity was measured as 517 nm as compared to standard α-tocopherol.

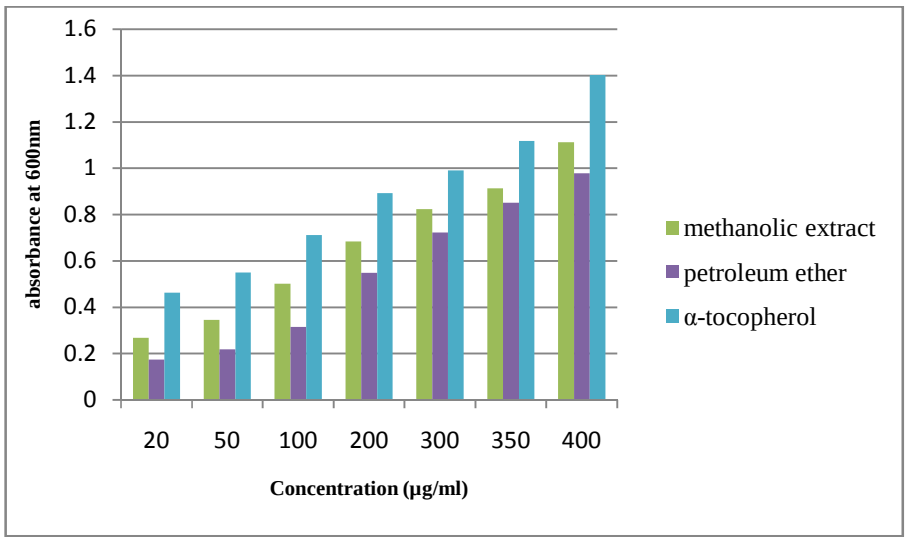

Figure 2: Reducing power of methanolic extract and petroleum ether extract of seeds of *Benincasa hispida* was compared to α-tocopherol.

When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. Comparison of the antioxidant activity of the extract and α-tocopherol is shown in Figure 1. The methanolic and petroleum ether extract of seeds of *Benincasa hispida*

exhibited a significant dose dependent inhibition of DPPH activity. The IC_{50} values of methanolic, petroleum ether extract and α-tocopherol were 5.167µg/ml, 6.157µg/ml and 4.15µg/ml respectively.

The reducing ability of a compound generally depends on the presence of reductants which have been exhibited antioxidative potential by breaking the Free radical chain, donating a hydrogen atom. The presence of reductants (i.e. antioxidants) in *Benincasa hispida* seeds extracts causes the reduction of the Fe 3+ /ferricyanide complex to the ferrous form. Therefore, the Fe 2+ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Figure 3, 4 shows the reductive capabilities of the *Benincasa hispida* seeds extracts compared to α-tocopherol. The reducing power of *Benincasa hispida* seeds extracts was very potent and the power of the extract was increased with quantity of sample. The reducing ability of methanolic extract is greater than petroleum ether extract as shown in figure 2.

CONCLUSION

It has been reported that reactive oxygen species contribute to various pathophysiological conditions and endogenous defense mechanisms have evolved to offer protection in these conditions. An increase in the antioxidant reserves of the organism can reduce oxidative stress and some of the plant derived agents may help to reduce it. Determination of the natural antioxidant compounds of plant extracts will help to develop new drug candidates for antioxidant therapy [5, 9]. The plants may be considered as good sources of natural antioxidants for medicinal uses such as against aging and other diseases related to radical mechanisms [2, 22, and 14]. Further investigation on the isolation and identification of antioxidant component(s) in the plant may lead to chemical entities with potential for clinical use.

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