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In vitro Evaluation of Antioxidant Activity of Methanolic and Petroleum Ether Extracts from Seeds 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 FTC (Ferric thiocyanate) and TBA (Thiobarbituric acid) methods. 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 percentage inhibition of linoleic acid by the methanolic extract and petroleum ether extract of seeds of B. hispida had shown effective results as compared to standard antioxidant. After seven days the percentage inhibitions of extracts were 65.43% and 51.08%. The percent inhibitions by extracts were comparable to α-tocopherol (77.61%). The TBA analysis of methanolic and petroleum ether extracts from the seeds of B. hispida at seventh day of storage. The percentage of antioxidant activity for methanolic and petroleum ether extracts were 71.02% and 63.65%. The results of extracts were comparable to α-tocopherol, where the percentage of activity was 83.72%. The results concluded that the extracts have a potential source of antioxidants of natural origin.

Key words: Antioxidant, *Benincas hispida*, free radical, FTC, TBA.

INTRODUCTION

Benincasa hispida occupies a very important place in the traditional medicinal system in India. *B. hispida* (Thomb.) commonly known as ash gourd belongs to the family of cucurbitaceae. It is employed as main ingredient in Kushmanda lehyam in Ayurvedic system of medicine [1]. The lehyam is used as a rejuvenative agent and in nervous disorder. Ash gourd is a vegetable widely used in India for various ailments such as gastrointestinal problems, respiratory diseases (cough, asthma), heart diseases, vermifuge, diabetes mellitus and urinary diseases [2; 3]. Tambussi [2000] and Warier [1994] suggested that *B. hispida* is useful for controlling of nervous disorders, ulcer healing and acid neutralizing. Kim and Shin [1999] and Grover *et al*., [2000] reported that expectorant effect of seed extract of *B. hispida* may be due to the mucus secretion which prevents gastric ulcer. *B. hispida* seed extract is also reported to enhance immunoreactions resulting in histamine secretion inhibition [8; 9].

In living systems, free radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics. In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting, can result in increased radical activity and damage. Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates

glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long-term complication of diabetes [10; 11; 12; 13].

Antioxidants are essential to good health. Diets rich in antioxidants contribute to a lower incidence of several major chronic diseases. In particular, cancer development and growth are inhibited by antioxidants. Antioxidants delay or prevent the oxidation of a given substrate by free radicals. Antioxidants are the substances that prevent the oxidation of cellular oxidizable compound by scavenging ROS, activating a battery of detoxifying protein or preventing generation of ROS [14]. Lipid peroxidation and protein/DNA oxidation studies document these activities. However, most activity studies focus on the relationship between flavonoid structure and antioxidant mechanism [15]. 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 product including polyphenolic substances (e.g., flavonoids and tannins) and various plant or herb extracts exert potent antioxidant actions [16; 17]. Several scientific reports have been reported on this activity in *Jatropha curcas* [18], sunflower [19], *Arabidopsis thaliana* [20], *Curcuma longa* [21], chickpea [22] and rapeseed [23], *Murraya koenigii* [24], *Solanum torvum* [25].

MATERIALS AND METHODS

Plant materials: Fruit of *B. hispida* was collected from Agra city of Uttar Pradesh in January 2012. Seeds were dried in hot air oven at 40°C for 48 hours.

Preparation of extract: Extracts were prepared according to [26].

Antioxidant assay: *In vitro* antioxidant activity of seed extracts of *B. hispida* were determined by FTC (Ferric thiocyanate) and TBA (Thiobarbituric acid) methods. All assays were carried out in triplicate and average value was considered.

Ferric thiocyanate (FTC) method:
The standard method described by [27] was used with some modification. 1 ml extract was mixed with 4ml of ethanol (99.5%) and the mixture was mixed with 4.1ml of 2.5% linoleic acid in 99.5% ethanol, 8.0 ml of 0.05 M potassium phosphate buffer (pH 7.0) and 3.9ml of water and the mixture placed in a screw capped vial then placed in an oven at 40˚C in the dark. 0.1ml of this solution was added to 9.7 ml of 75% ethanol and 0.01 ml of 30% ammonium thiocyanate. Then the 0.2 ml of 0.02 M ferrous chloride in 3.5% HCl added to the mixture. The absorbance of red color was measured at 500 nm by using UV visible spectrophotometer every 24 h, till the absorbance of control reached maximum (day seven). α-tocopherol was used as positive control while the mixture without sample was used as the negative control.

The inhibition of lipid peroxidation was calculated as follows:

$$\% \text{ inhibition} = (A_o - A_1/A_o) \times 100$$

A_o is the absorbance of the control reaction and A_1 is the absorbance in the presence of the extract.

Thiobarbituric acid (TBA) test:
The TBA test was conducted according to the method of [28; 29]. According to this method 2 ml of 20% tricholoacetic acid and 2ml of 0.67% of 2-thiobarbituric acid was added to 1ml of extract which was prepared by FTC method. This mixture was then placed in boiling water bath at 100˚C for 10 minutes. After cooling it was centrifuged at 3000 rpm for 20 minutes at normal temperature. The absorbance of the supernatant was measured at 532 nm using UV visible spectrophotometer. The mixture without added sample was used as control and α-tocopherol was used as the standard.

The antioxidant activity was calculated as percentage of inhibition in this method as follows:

$$\% \text{ inhibition} = (A_o - A_1/A_o) \times 100$$

A_o is the absorbance of the control reaction and A_1 is the absorbance in the presence of the extract.

RESULTS AND DISCUSSION

In this present study the antioxidant activity of the methanolic and petroleum ether extracts of seeds *B. hispida* were investigated by using FTC (Ferric thiocyanate) and TBA (Thiobarbituric acid) methods. All methods have proven the effectiveness of the methanolic and petroleum ether extract compared to the standard antioxidant α- tocopherol (Vitamin E). The FTC method measures the amount of peroxide value in the beginning of the liquid peroxidation, where ferric ion is formed upon reaction of peroxide with ferrous chloride. The ferric ion then unites with ammonium thiocyanate producing ferric thiocyanate, a red colored substance. The darker the color, the higher will be the absorbance. From the analysis it shows that all samples had been oxidized when stored for seven days at 40-45˚C. Initially the percentage inhibition of extract was low. After seven days the percentage inhibition of methanolic and petroleum ether extracts were 65.43% and 51.08%, whereas the percentage inhibition of α- tocopherol was 77.61%.

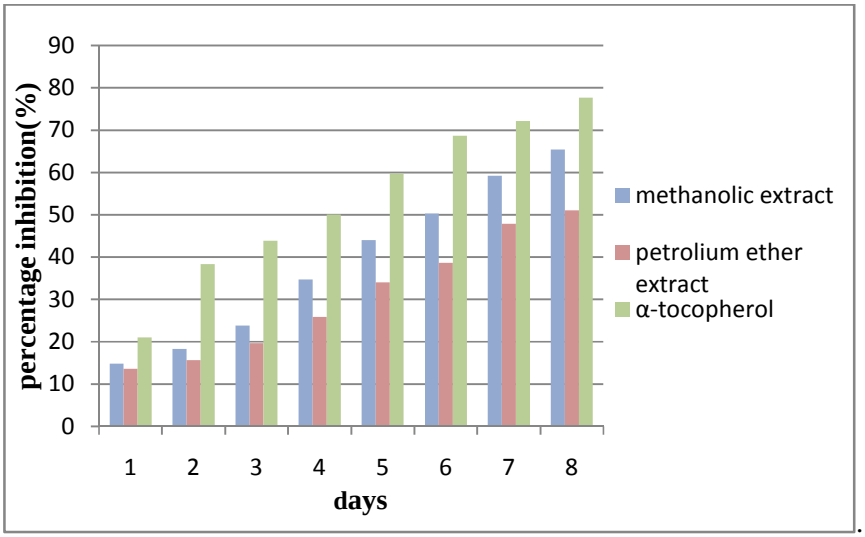

Figure 1: Antioxidant activity of methanolic and petroleum ether extracts of seeds of *B. hispida* as measured by the FTC method at 500 nm and compared to standard α-tocopherol.

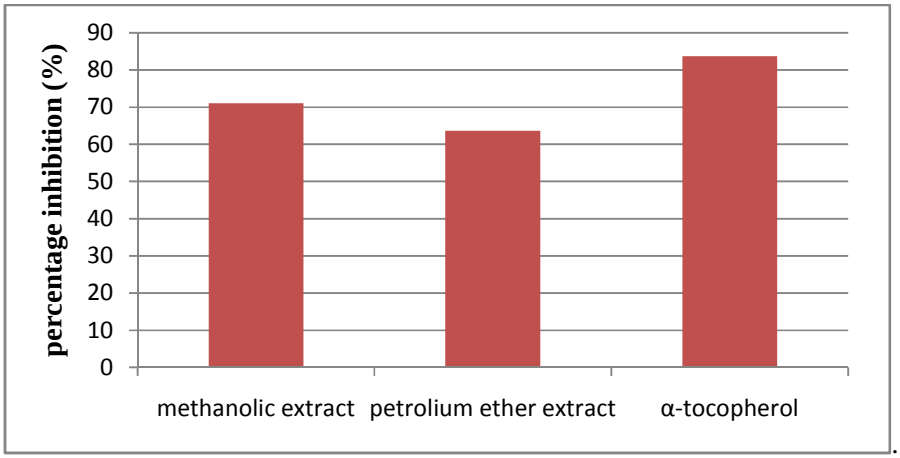

Figure 2: Antioxidant activity of methanolic and petroleum ether extracts of seeds of *B. hispida* as measured by the TBA method at 532 nm and compared to standard α-tocopherol.

FTC used to measure the production of peroxide compound at the initial stage of oxidation while TBA test is used to measure the secondary product of oxidation such as aldehyde and ketone. The TBA analysis of the methanolic and petroleum ether extracts from the seeds of *B. hispida* at seventh day of storage. The percentage of antioxidant

activity for methanolic and petroleum ether extracts were 71.02% and 63.65%. The results extracts were comparable to α-tocopherol, where the percentage of activity was 83.72%.

CONCLUSION

From the above results it can be conclude that methanolic extract of seeds of *B. hispida* showed more potent, *in vitro* antioxidant activity with high percent of inhibition as compare to petroleum ether extract. The results show high antioxidant activity in terms of oxidation inhibition and free radical scavenging, thus indication possible benefits to human health when present in the diet. Natural antioxidant compounds of seed extracts can help to develop new drugs for antioxidant therapy.

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