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In vitro antioxidant activity studies on leaves of *Benincasa hispida* (Thunb.) Cogn.

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ABSTRACT

Benincasa hispida is an annual climber and its fruits are used in the management of a host of medical problems, including epilepsy and lung diseases. In the present study, *in-vitro* antioxidant activity of hexane, ethyl acetate and methanolic extracts of *B. hispida* leaves were investigated by using Superoxide radical, Hydroxyl radical and DPPH radical scavenging methods. The tested extracts showed dose-dependent inhibition of *in vitro* free radical generation of superoxide anion, hydroxyl radical and DPPH radicals. The results clearly revealed free radical scavenging activity of the tested leaf extracts of *B.hispida* and this activity is compared with that of the standard drug ascorbic acid.

Keywords: Antioxidant activity*, Benincasa hispida* leaf extracts.

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INTRODUCTION

Benincasa hispida belongs to Cucurbitaceae family and its origin can be traced to south-east Asia. It is a very popular vegetable in India, China, Philippines, Thailand, Vietnam and in some non-Asian countries [1]. It is traditionally used in treatment of several problems including epilepsy, lung diseases, asthma, cough, urine retention and internal hemorrhage. It is an excellent treatment for tapeworms disinfection and useful for controlling of nervous disorders, ulcer healing and acid neutralizing [2,3]. Seed extracts shown to prevent gastric ulcers [4]. It was shown to enhance immunoreactions leading to histamine secretion inhibition [5], [6]. Since no work has been published on the *in vitro* antioxidant activity of the of *B.hispida* a detailed study has been carried out on the extracts of *B.hispida* leaves for the scavenging activity of superoxide radical, Hydroxyl radical and DPPH radicals.

MATERIAL AND METHODS

Plant Extraction

The plant material (*Benincasa hispida*) was collected in and around Visakhapatnam region, Andhra Pradesh, India. The freshly collected leaves of the plant were shade dried and powdered. The powdered material (5 kg) was subjected to successive soxhlet extraction with hexane, ethyl acetate and methanol. The solvents thus obtained were separately concentrated under vacuum at temperature of 55 ºc by using rotavapor. The concentrated extracts were collected and stored in desiccators for further use.

Superoxide Radical Scavenging Activity

Superoxide scavenging activity of the plant extract was determined by McCord and Fridovich method, 1969 [7]. 0.1ml of different concentrations of plant extract were mixed with 0.1 ml of 6 µM ethylenediamine tetra-acetic acid and transferred to a test tube, and final volume was made up to 3 ml using phosphate buffer. The assay tubes were uniformly illuminated with an incandescent light (40 Watts) for 15 minutes and thereafter the optical densities were measured at 560 nm. A control was prepared by using 0.1 ml of respective vehicle with ascorbic acid. The percentage inhibition of superoxide production was evaluated by comparing the absorbance values of control and experimental tubes. The percentage inhibition of superoxide production by the extract was calculated using the following formula:

$$\text{Inhibitory ratio} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A_0 is the absorbance of control (ascorbic acid).; A_1 is the absorbance with addition of plant extract

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe2+/EDTA/H2O2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances [8]. The thiobarbituric acid reactive substance was measured at 532 nm. A control was prepared using 0.1 ml of respective vehicle with ascorbic acid. The percentage inhibition of hydroxyl radicals by the extracts was determined by comparing the absorbance values between the control and the respective plant extracts.

DPPH Radical Scavenging Activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by the Braca *et al*., 2003 [9]. In the assay, the DPPH reagent was prepared by dissolving the 0.04 g DPPH in 100ml of methanol. From this stock 3 ml of DPPH solution in methanol and 0.1 ml of plant extract at various concentrations (100-1300 µg/ml) were mixed. The mixture vortexed and allowed for 30 min to reach a steady state at room temperature. Decolonization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract

The percentage inhibition activity was calculated as extract/ascorbic acid.

$$\text{Inhibitory ratio} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A_0 is the absorbance of control (ascorbic acid); A_1 is the absorbance of the plant extract

RESULTS AND DISCUSSION

Superoxide Radical Scavenging Activity

Superoxides are produced from molecular oxygen due to oxidative enzymes of body as well as via non- enzymatic reactions such as auto-oxidation by catecholamine [10]. In the present study, the different extracts of *B.hispida* showed concentration dependent scavenging activity on superoxide radicals (Fig.1). The standard drug ascorbic acid showed better percentage of inhibition of superoxide radical than the samples of tested species. The mean IC_{50} values for superoxide radical of BHHE, BHEA and BHME of *B. hispida* were found to be 947µg, 532µg and 128µg respectively. The mean IC_{50} value of standard ascorbic acid was found to be 56.5µg. The results are summarized in Table-1 and Fig.2.

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Hydroxyl Radical Scavenging Activity

Among the reactive oxygen species, the hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism [11]. In this study, the tested extracts (BHHE, BHEA & BHME) showed concentration- dependent scavenging activity on hydroxyl radicals as depicted in Fig.3. Among the extracts methanolic extract showed better percentage of inhibition. The mean IC_{50} values for hydroxyl radical of BHHE, BHEA and BHME of *B.hispida* were found to be 513µg, 353µg and 235µg respectively. The mean IC_{50} value of ascorbic acid for hydroxyl radical was found to be 69.4µg. The IC_{50} values were summarized in Table-1 & Fig.2.

Table 1: In-vitro 50% inhibition concentration (IC_{50}) of different extracts of *Benincasa hispida* on DPPH, Superoxide and Hydroxyl free radicals.

Name of the extract	IC_{50} value (µg)		
	Superoxide radical	Hydroxyl radical	DPPH radical
BHHE	947 ± 0.16	513 ± 0.12	1094 ± 0.11
BHEA	532 ± 0.12	353 ± 0.11	471 ± 0.11
BHME	128 ± 0.15	235 ± 0.23	182 ± 0.23
Ascorbic acid	56.5 ± 0.21	69.4 ± 0.22	18.7 ± 0.18

BHHE: *Benincasa hispida hexane extraction*; **BHEA:** *Benincasa hispida ethyl acitate;* **BHME:** *Benincasa hispida methanol extraction*

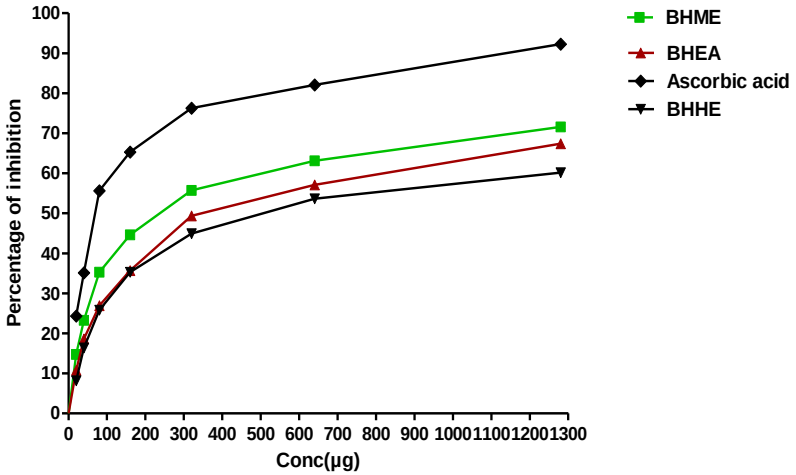

Figure 1: Concentration dependent percent inhibition of Superoxide radical by different extracts of *Benincasa hispida* and Ascorbic acid

DPPH Radical Scavenging Activity

The free radical scavenging activity of the selected plant extracts was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH [12], [13].

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DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with both DPPH transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1-1-diphenyl -2 -picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug [14]. The tested extracts showed concentration dependent scavenging activity of DPPH radicals as depicted in Fig.4. The mean IC_{50} values for DPPH radical of BHHE, BHEA and BHME of *B.hispida* were found to be 1094µg, 471µg and 182µg respectively. The mean IC_{50} value of ascorbic acid was found to be 18.7µg. The IC_{50} values were summarized in Table-1 & Fig.2

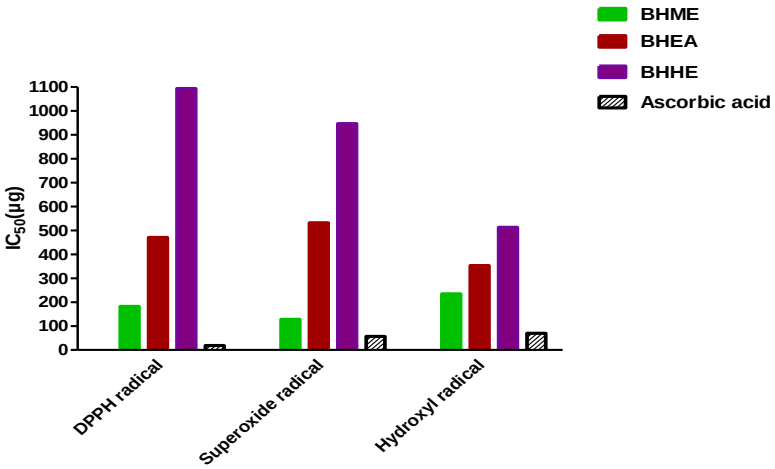

Figure 2: In vitro 50% inhibition concentration (IC50) of different extracts of *Benincasa hispida* on DPPH, Superoxide and Hydroxyl free radicals.

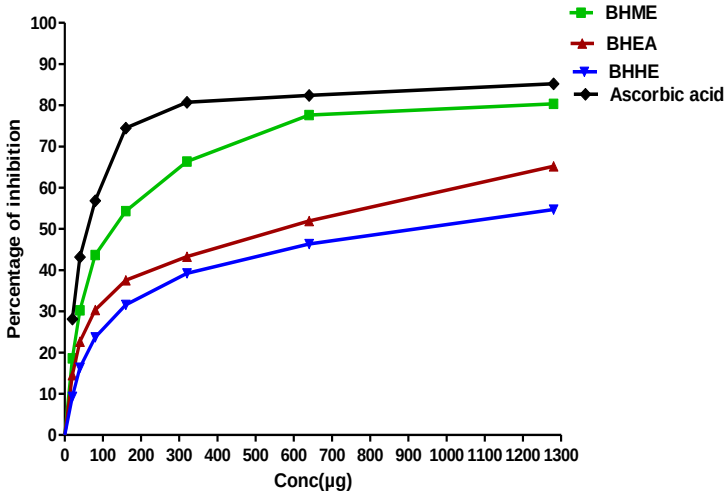

Figure 3: Concentration dependent percent inhibition of Hydroxyl radical by different extracts of *Benincasa hispida* and Ascorbic acid

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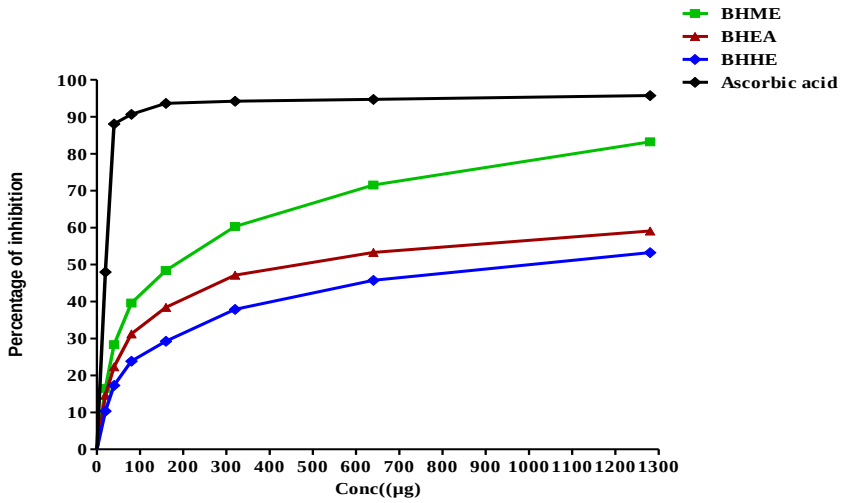

Figure 4. Concentration dependent percent inhibition of DPPH radical by different extracts of *Benincasa hispida* and Ascorbic acid

The order of free radical scavenging activity of the three extracts (BHHE, BHEA & BHME) of *B.hispida* leaves against the three tested radicals (superoxide, hydroxyl and DPPH radicals) was in the following order: Ascorbic acid> BHME>BHEA>BHHE. The results indicate methanolic extract of *B.hispida* leaves shown better antioxidant activity. The better antioxidant activity of *B.hispida* leaves in methanolic extract may be attributable to the maximum solubility of several antioxidant phytochemicals like flavonoids, Saponins, alkaloids, phenols etc., in methanol.

CONCLUSIONS

In the present study, concentration dependent inhibition of the three tested free radicals was observed with the hexane, ethyl acetate, methanol extracts of *B.hispida* leaves. The results indicate that methanolic extract of *B.hispida* leaves shown better antioxidant activity when compared to hexane and ethylacetate extracts on the three tested radicals.

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