

## In Vitro Antioxidant Activity Studies On Leaves Of Cucurbita Maxima

Bhavani Yenda<sup>1\*</sup>, Bandaru V Rao<sup>1</sup> and B Ganga Rao<sup>2</sup>

<sup>1</sup>Department of Botany, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India.

<sup>2</sup>College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India.

\*Corresponding Author's Email: bhavaniyenda@gmail.com

### ARTICLE INFO

#### Article history:

Received 12 Nov. 2014

Accepted 30 Dec. 2014

Available online 06 Jan. 2014

#### Keywords:

Antioxidant activity,  
Cucurbita maxima,  
leaf extracts,  
in vitro

### ABSTRACT

In this study, In-vitro antioxidant activity of hexane (CMHE), ethyl acetate (CMEA) and methanolic (CMME) extracts of *Cucurbita maxima* leaves was investigated by using Superoxide radical, Hydroxyl radical and DPPH radical scavenging methods. *C. maxima* is an annual climber growing up to 5 m. It is mentioned in Ayurveda as a medicinal plant for the treatment of tapeworms, wounds etc. The tested extracts showed dose dependent inhibition of *in vitro* free radical generation of superoxide anion, hydroxyl radical and DPPH radicals. The results clearly indicated the free radical scavenging activity of tested extracts of *C. maxima*, this activity is comparable with that of the standard drug ascorbic acid.

© 2015 International Journal of Advanced Research in Science and Technology (IJARST).

All rights reserved.

### Introduction:

*Cucurbita maxima* belongs to the family Cucurbitaceae is traditionally used in treatment of several problems of stomach, wounds and tapeworms. *C. maxima* is native of Malaysia but it is now found throughout the tropical countries. It is distributed throughout India, China, Burma, Thailand and Vietnam and is widely spread throughout the tropical and subtropical countries of the world. Pumpkin is one such plant that has been frequently used as functional food or medicine. *C. maxima* reported to have antidiabetic<sup>(1)</sup>, hepatoprotective<sup>(2)</sup>, anthelmintic<sup>(3)</sup>, immunomodulatory, antihypertensive, anticancer, antibacteria, antihypercholesterolemic and antinflammatory<sup>(4)</sup> activities. The plant is reported to have triglyceride fatty acid mixture, tetrahydro-thiophene, linolic acid, calotropoleanyl ester, cholesterol and 13(18)-oleanen-3-ol<sup>(5)</sup>. Proteins from pumpkin seeds were reported to inhibit melanoma proliferation<sup>(6)</sup>. Flavonoids, tannins and other phenolic constituents present in food of plant origin are also potential antioxidants<sup>(7-8)</sup>. However, literature survey indicated no published reports on the *in vitro* antioxidant activity of the successive extracts of *C. maxima*. Hence, a detailed study was carried out on the hexane (CMHE), ethyl acetate (CMEA) and methanolic (CMME) extracts of *C. maxima* leaves for the scavenging activity of superoxide radical, Hydroxyl radical and DPPH radicals.

### Material and methods Plant extraction:

The plant material (*Cucurbita maxima*) 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 then subjected to successive soxhlet extraction with hexane, ethyl acetate and methanol. The solvents thus obtained were separately concentrated under vacuum at temperature of 55 °c 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 & Fridovich, 1969 method<sup>(9)</sup>. 0.1ml of different concentrations of plant extract were mixed with 0.1 ml of 6 µM ethylenediamine tetraacetic 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  $Fe^{2+}/EDTA/H_2O_2$  system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances <sup>(10)</sup>. The thiobarbituric acid reactive substances 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 Braca et al., 2003<sup>(11)</sup>. In the assay, the DPPH reagent was prepared by dissolving the 0.04 g DPPH in 100 ml of methanol. From the stock 3ml of DPPH solution in methanol and 0.1 ml of plant extract at various concentrations [100 to 1300 $\mu$ g/ml] were mixed. The mixture vortexed and allowed for 30 min to a steady state at room temperature. Decolorization 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:**

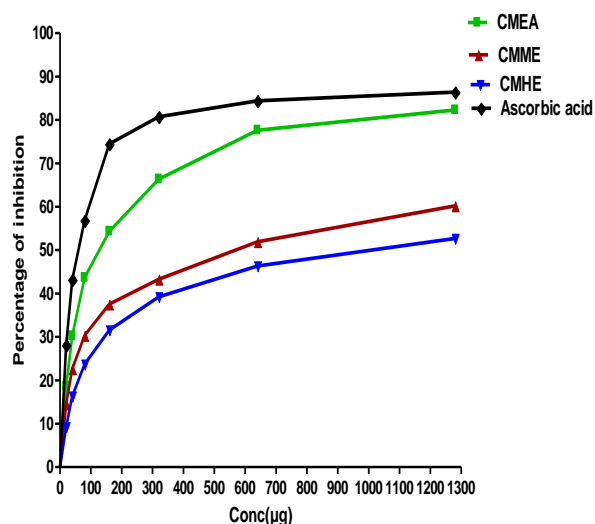
**Superoxide Radical Scavenging Activity:**

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 <sup>(12)</sup>. In the present study, the different extracts of *C.maxima* 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 CMHE, CMEA and CMME of *C.maxima* were found to be 895 $\mu$ g, 131 $\mu$ g and 528 $\mu$ g respectively. The mean  $IC_{50}$  value of standard ascorbic acid was found to be 56.5. The  $IC_{50}$  values were summarized in Table-1 and Fig.2.

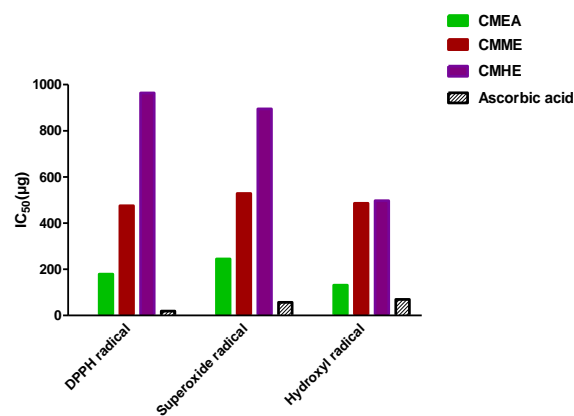
**Table: 1.** In-vitro 50% inhibition concentration ( $IC_{50}$ ) of different extracts of *Cucurbita maxima* on DPPH, Superoxide and Hydroxyl free radicals.

Name of the extract of <i>Cucurbita maxima</i>	$IC_{50}$ value ( $\mu$ g)		
	Superoxide radical	Hydroxyl radical	DPPH radical
CMHE	895	497	964
CMEA	131	245	179
CMME	528	486	475
Ascorbic acid	56.5	69.4	18.7

CMHE: cucurbita maxima hexane extraction,  
 CMEA: cucurbita maxima ethylacetate,  
 CMME: cucurbita maxima methanol extraction.



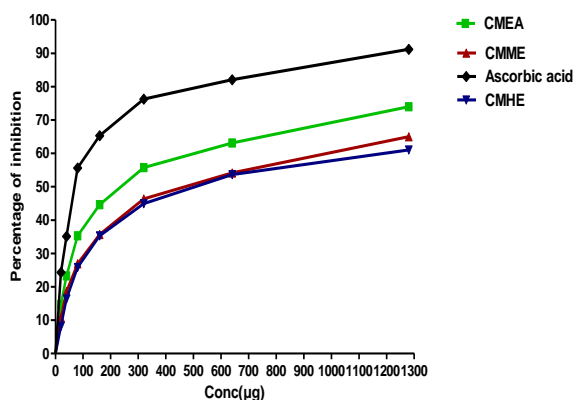
**Fig.1:** Concentration dependent percent inhibition of Superoxide radical by different extracts of *Cucurbita maxima* and Ascorbic acid.



**Fig.2:** In vitro 50% inhibition concentration ( $IC_{50}$ ) of different extracts of *Cucurbita maxima* on DPPH, Superoxide and Hydroxyl free radicals.

### Hydroxyl Radical Scavenging Activity:

Among the reactive oxygen species, the hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism<sup>[13]</sup>. In this study, the tested extracts (CMHE, CMEA & CMME) showed concentration dependent scavenging activity on hydroxyl radicals as depicted in Fig.3. Among the extracts ethylacetate extract showed better percentage of inhibition. The mean IC<sub>50</sub> values for hydroxyl radical of CMHE, CMEA and CMME of *C.maxima* were found to be 497μg, 245μg and 486μg respectively. The mean IC<sub>50</sub> value of ascorbic acid for hydroxyl radical was found to be 69.4μg. The IC<sub>50</sub> values were summarized in Table-1 and Fig.3.



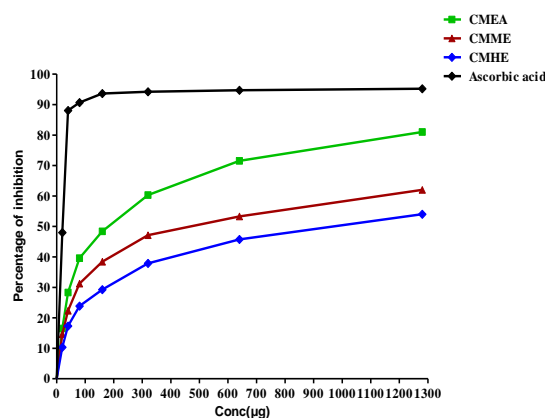
**Fig.3:** Concentration dependent percent inhibition of Hydroxyl radical by different extracts of *Cucurbita maxima* and Ascorbic acid.

### DPPH Radical Scavenging Activity:

Free radical scavenging activity of the three extracts (CMHE, CMEA & CMME) of *C.maxima* leaves against the three tested radicals (superoxide, hydroxyl and DPPH radicals) was in the following manner : an The order of DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH both 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<sup>[14]</sup>. The tested extracts showed concentration dependent scavenging activity of DPPH radicals as depicted in Fig 4. The mean IC<sub>50</sub> values for DPPH radical of CMHE, CMEA and CMME of *C.maxima* were found to be 964μg, 179μg and 475μg respectively. The mean IC<sub>50</sub> value of ascorbic acid was found to be 18.7μg. The IC<sub>50</sub> values were summarized in Table-1 and Fig.2.

Ascorbic acid > CMEA > CMME > CMHE. The results indicate ethylacetate extract of *c.maxima* leaves shown better antioxidant activity. The better antioxidant activity of *c.maxima* leaves in ethylacetate may be attributable to the maximum solubility of several

antioxidant phytochemicals like flavonoids, saponins, alkaloids, phenols etc., in ethylacetate.



**Fig.4:** Concentration dependent percent inhibition of DPPH radical by different extracts of *Cucurbita maxima* and Ascorbic acid.

### Conclusion:

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

### Acknowledgment:

The first author is grateful to for granting Junior Research Fellowship under U.G.C Special Assistance Programme.

### References:

- Sharma A, Sharma AK, Chand T, Khardiya M, Yadav KC. Antidiabetic and antihyperlipidemic activity of *Cucurbita maxima* Duchense (pumpkin) seeds on streptozotocin induced diabetic rats. *J Pharmacog Phytochem.* 2013; 1(6): 108-116.
- Saha P, Mazumder UK, Haldar PK, Bala A, Kar B, Naskar S. Evaluation of hepatoprotective activity of *Cucurbita maxima* aerial parts. *J Herbal Med Toxicol.* 2011; 5(1): 17-22.
- Saha P, Mazumder UK, Haldar PK, Naskar S, Kundu S, Bala A, Kar B. Anticancer activity of methanol extract of *Cucurbita maxima* against Ehrlich ascites carcinoma. *Int. J. Res. Pharm. Sci.* 2011; 2(1): 52-59.
- Iqbal Z, Nadeem QK, Khan MN, Akhtar MS, Waraich FN. In Vitro Anthelmintic activity of *Allium sativum*, *Zingiber officinale*, *Curcubita mexicana* and *Ficus religiosa*. *Int J Agri Biol.* 2001; 3(4): 454-45.
- Badr-Sherif EA, Shaaban M, Elkholy YM, Helal MH, Hamza AS, Masoud MS, Safty MME. Chemical composition and biological activity of ripe pumpkin fruits (*Cucurbita pepo*L.) cultivated in Egyptian habitats. *Nat Prod Res.* 2011; 25(16): 1524-1539.
- Xie, J.M. 'Induced polarization effect of pumpkin protein on B16 cell'. *Fujian Med Univ Acta*, vol.38, no.4, 2004 pp.394-395.
- Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP and Rice-Evans CI. Polyphenolic flavanols as scavengers

- of aqueous phase radicals and as chain breaking antioxidants. *Archives of Biochemistry and Biophysics* 1995;322(2):339-346.
8. Van Acker SABE, Van den Vijgh WJF and Bast F. Structural aspects of antioxidant activity of flavonoids. *radical Biology and Medicine* 1996; 20(3): 331-342.
  9. Mc Cord JM, Fridovich I. Superoxide dismutase: an enzymic function for erythrocyte hemocuprein. *J Biol Chem* 1969; 244: 6049-55.
  10. Walling C. Fenton's reagent revisited. *Acc Chem Res* 1975; 8:125-31.
  11. Braca A, Fico G, Morelli I, De Simone F, Tome F, De Tommasi N. Antioxidant and free radical scavenging activity of flavonol glycosides from different *Aconitum* species. *J Ethnopharmacol* 2003; 86:63-67.
  12. Elizabeth Kunchandy, Rao MNA. Oxygen radical scavenging activity of curcumin. *Int J Pharm* 1990, 58:237-40
  13. Gino A Kurian, Tejeshvi Rajamani, Pavithra Ramanarayanan, Jose Paddikkala. A comparative study on in vitro and in vivo antioxidant activities of aqueous extract of *Desmodium gangeticum* (Luguminosae) root. *Int J Green Pharm* 2009; 324-31.
  14. Fazel Shamsa, Hamidreza Monsef, Rouhollah Ghamooshi, Mohammadreza Verdian-rizi. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Thai J. Pharm. Sci* 2008; 32: 17-20.