In Vitro Antioxidant Activity of *Cucurbita Maxima* Aerial Parts

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ABSTRACT

Introduction: Free radical induced oxidative stress is involved in the pathogenesis of various diseases and disorders. Antioxidants play an important role against this oxidative stress to protect our body. The present study was carried out to evaluate the *in vitro* antioxidant properties of methanol extract of *C.maxima* aerial parts (MECM). **Methods:** MECM was assayed on different *in vitro* free radical models like, DPPH, nitric oxide, superoxide, hydrogen peroxide and lipid peroxide radical models. Reductive ability of the extract was also tested by the complex formation with potassium ferricyanide. Further total phenolic and flavonoid contents of the crude extract were also measured. Butylated hydroxy toluene was taken as standard. **Result:** The extract showed good dose dependent free radical scavenging activity in all the models. Reductive ability was also found to increase with increase in extract concentration. Determination of total phenolic and total flavonoids content showed that 1 gm of dry extract contains 66.70±3.60 mg equivalent of pyrocatechol and 26.50±1.40 mg equivalent of quercetin. **Conclusion:** All the results of the *in vitro* antioxidant assays revealed potent antioxidant and free radical scavenging activity of the aerial part of *C.maxima*, equivalent to that of standard BHT and this antioxidant property may be attributed to its high phenolic and flavonoid contents.

Keywords: *Cucurbita maxima*, Cucurbitaceae, DPPH, free radicals, Lipid peroxidation, oxidative stress.

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INTRODUCTION

Oxygen derived free radicals such as superoxide, hydrogen peroxide, hydroxyl radicals are collectively known as reactive oxygen species (ROS).During normal physiologic condition, ROS are continuously produced in the aerobic cells and removed by endogenous antioxidant defense mechanism of the cell. But, under pathologic condition the balance between ROS and antioxidant defense mechanism is lost.[1] Overproduction of ROS and other free radicals then can damage cellular proteins, carbohydrates, lipids and DNA and may thus lead to oxidative stress which in turn results in a variety of diseases, such as liver cirrhosis, inflammation, atherosclerosis, diabetes, cancer, neurodegenerative disease, nephrotoxicity and also the aging process. Antioxidants have the potential to prevent these oxidative damages and thereby minimize the homeostatic disturbances.[2-6]

Many plants contain substantial amounts of antioxidants and can be utilized to scavenge the excess free radicals. The protection offered by different edible plants against oxidative stress in several diseases has been attributed to various antioxidants and vitamins. Potential antioxidant properties of the dietary phenolic compounds and flavonoids present in various fruits and vegetables have recently been recognized in a number of investigations.[7,8]

The plant *Cucurbita maxima* Duchesne (commonly known as pumpkin) belongs to the family Cucurbitaceae and is widely cultivated throughout the world for use as vegetable as well as medicine. Both of its fruits and the aerial parts are commonly consumed as vegetable. It is a large climbing herb, annual or perennial. Its aerial part consists of flexible succulent stem with trifoliate leaves. [9] The plant has been used traditionally as medicine in many countries such as China, India, Yugoslavia, Brazil and America.[10-12] Traditionally it is used in most countries as antidiabetic, antitumor, antihypertensive, anti-inflammatory, immunomodulatory and antibacterial agents.[13] Several *in vitro* and *in vivo* studies with crude pumpkin fruit extract as well as various purified fractions, including proteins and polysachharides, have explored its antitumor, antidiabetic and other medicinal values.[10,14] Popularity of pumpkin in various traditional system of medicine for several ailments focused the investigators'

attention on this plant. The present study was therefore carried out to investigate the antioxidant potential of methanol extract of *C. maxima* aerial parts (MECM) on different *in vitro* models.

MATERIALS AND METHODS

Chemicals

1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Chemicals, USA. Nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), reduced nicotinamide adenine dinucleotide (NADH), sodium nitroprusside, napthyl ethylene diamine dihydrochloride, ascorbic acid, trichloroacetic acid (TCA), thiobarbituric acid (TBA), potassium ferricyanide [$K_3Fe(CN)_6$] , Folin-Ciocalteu's phenol reagent (FCR) were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. All other chemicals and solvents used were of analytical grade.

Plant Material

The aerial parts of *C.maxima* were collected in June 2009, from Khardah, West Bengal, India and identified by the Botanical Survey of India, Howrah, India. A voucher specimen (P/CM/2/09) was retained in our laboratory for further reference.

Preparation of Plant Extract

The aerial parts were dried and powdered in a mechanical grinder. The powdered material was extracted with methanol using soxhlet apparatus. This extract was filtered and concentrated in *vacuo* in a Buchi evaporator, R-114 and kept in a vacuum dessicator until use. The yield was 11.49% w/w with respect to dried powder. This methanol extract was used for the present study.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out by following the standard procedures.[15]

In vitro Antioxidant Studies

Various concentrations of MECM (10-320 μg/ml in methanol) were used for the antioxidant studies on different *in vitro* models. For reductive ability study, 100-800 μg/ml concentration of the extract was used. Butylated hydroxy toluene (BHT) was used as standard.

Determination of DPPH Radical Scavenging Activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was measured using the method of Cotelle *et al*[16] with some modifications. 3 ml of reaction mixture containing 0.2 ml of DPPH (100 μM in methanol) and 2.8 ml of test or standard solution of various concentrations was incubated at 37°C for 30 min and absorbance of the resulting solution was measured at 517 nm using Beckman model DU-40 spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the formula:

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

Determination of Nitric Oxide (NO) Scavenging Activity

At physiological pH, sodium nitroprusside generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be measured by the Griess reaction[17]. 1ml of 10 mM sodium nitroprusside was mixed with 1 ml of test or standard solution of different concentrations in phosphate buffer (pH 7.4) and the mixture was incubated at 25°C for 150 min. From the incubated mixture, 1 ml was taken out and 1 ml of Griess' reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added to it. Absorbance of the chromophore formed by the diazotization of nitrite with sulfanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was read at 546 nm and percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Determination of Superoxide (SO) Radical Scavenging Activity

Superoxide anion scavenging activity was measured according to the method of Robak and Gryglewski[18] with some modifications. All the solutions were prepared in 100 mM phosphate buffer (pH 7.4). 1ml of nitroblue tetrazolium (NBT, 156 μM), 1 ml of reduced nicotinamide adenine dinucleotide (NADH, 468 μM) and 3 ml of test/ standard solution were mixed. The reaction was initiated by adding 100 μl of phenazine methosulphate (PMS, 60 μM). The reaction mixture was incubated at 25°C for 5 min,