

THIN LAYER CHROMATOGRAPHIC STUDIES AND *IN VITRO* FREE RADICAL SCAVENGING EFFECTS OF *CUCURBITA MAXIMA* LEAF EXTRACTS

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Summary

Cucurbita maxima Duchesne (Cucurbitaceae), commonly known as pumpkin, is commonly grown vegetable and also cultivated worldwide for its consumable fruits. The present study assessed the different solvent extracts of *C. maxima* leaf for thin layer chromatography (TLC) and also evaluated their *in vitro* free radical scavenging potential by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay. The petroleum ether extract yielded maximum spots in TLC. All of the extracts exhibited potent *in vitro* free radical scavenging activity that increased with extract concentration. The methanol extract was found to be the most potent in this regard, followed by the chloroform and petroleum ether extracts.

Key words: Free radical scavenging, leaf, antioxidant, *Cucurbita maxima*.

Introduction

Antioxidants protect living organisms from damage caused by uncontrolled production of reactive oxygen species (ROS) and the concomitant lipid peroxidation, protein damage and DNA strand breaking. Current interest is focused on the potential role of antioxidants and antioxidant enzymes in the treatment and prevention of atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus and several others diseases (1).

Antioxidants are added to a variety of foods to prevent free radical induced lipid peroxidation, which is responsible for the development of off-flavors and the undesirable chemical compounds in food (2). These ROS cause destructive and irreversible damage to the components of a cell, such as lipids, proteins DNA and other macromolecules. Although normal cells possess antioxidant defense systems against ROS in the cells induces diseases such as cancer and aging (3).

ROS are formed and degraded by all aerobic organisms. ROS can readily react with most biomolecules including proteins, lipids, lipoproteins and DNA. Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive oxygen species, which are capable of oxidizing biological molecules, resulting in tissue damage and cell death. When the mechanism of antioxidant protection becomes unbalanced by exogenous and endogenous factors, it results in inflammation, diabetes, genotoxicity, cancer and accelerating aging (4).

Antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage. The most commonly used antioxidants are BHA, BHT, propyl gallate and tert-butyl-hydroquinone (5). However, they have been suspected of being responsible for liver damage and carcinogenesis in laboratory animals. Therefore, the development and use of more effective antioxidants is desired.

Traditional medicine worldwide is being reevaluated by extensive research on different plant species and their therapeutic principles. Plants produce antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity.

The plant *Cucurbita maxima* Duchesne, commonly known as pumpkin belongs to the family Cucurbitaceae and is widely cultivated throughout the world for use as culinary 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 trifoliolate leaves (6). The plant has been used traditionally as medicine in many countries such as China, India, Yugoslavia, Brazil and America (7-9). Traditionally it has been used in most countries as anti-diabetic, antitumor, antihypertensive, anti-inflammatory, immunomodulatory and antibacterial agents (10). Several *in vitro* and *in vivo* studies with crude pumpkin fruit extract as well as various purified fractions, including proteins and polysaccharides, have shown anticancer activity against melanoma, Ehrlich ascites carcinoma and leukaemia (11). Proteins from pumpkin seeds were reported to inhibit melanoma proliferation (12). However, in spite of traditional use, pharmacology of its aerial parts has not yet been explored scientifically. In the present study, we have aimed to evaluate *in vitro* free radical scavenging activity of different extracts from *C. maxima* leaf against 1, 1-diphenyl-2-picryl-hydrazil.

Materials and methods

Plant material: The mature leaves of *Cucurbita maxima* Duchesne (Cucurbitaceae) were collected during September 2011 from New Alipore, Kolkata, West Bengal, India. The plant material was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen [CNH/98/2011/Tech II/594] was maintained in our research laboratory for future reference. The plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

Preparation of plant extracts: The dried powdered material was defatted with petroleum ether (60-80°C), the percentage extractive value was 1.33 % w/w. The defatted powdered material thus obtained was further extracted with chloroform and methanol for 72 h. The solvent was distilled off in reduced pressure and resulting semisolid mass was vacuum dried to yield the dry extracts and the percentage extractive values were accordingly 2.42 % w/w and 6.54 % w/w respectively. The preliminary phytochemical analysis was performed for all three extracts to identify the phytoconstituents present in the extracts (13).

Chemicals: L ascorbic acid (vitamin C) and 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade obtained commercially.

Thin layer chromatographic studies: Each solvent extract was subjected to thin layer chromatography (TLC) as per standard one dimensional ascending method. The results and chromatograms are depicted in Figures 1-3.

Free radical scavenging activity measured by 1, 1-diphenyl-2-picryl-hydrazil: The free radical scavenging activity of all of the extracts were measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH) using the reported method (14). Briefly, an 0.1 mM solution of DPPH in methanol was prepared, and 1 ml of this solution was added to 3 ml of the all of the extracts solution respectively in petroleum ether, chloroform and methanol at different concentrations (2, 4, 6, 8, 10, 15 µg/ml). The mixture were shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Genesys 10 UV: Thermo Electron Corporation). Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. Ascorbic acid at same concentrations was used as reference. The capability to scavenge the DPPH radical was calculated by using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance of presence of all of the extract samples and reference. The results are stated in Table 1.

Results and discussion

Preliminary phytochemical studies showed the presence of steroids in the petroleum ether extract, triterpenoids in chloroform extracts whereas triterpenoids, tannins, glycosides and carbohydrates in the methanol extract form *C. maxima* leaf.

Among the various methods for separating plant constituents, the chromatographic procedure is the one of the most commonly used techniques of general application (15). Thin layer chromatography (TLC) involves the separation of mixtures of organic compounds on thin layers of adsorbents that are usually coated on glass, plastic, or aluminum sheets; and this particular technique is the easiest, cheapest and most widely used method for the characterization of natural products and their preparations (16).

The present thin layer chromatographic studies revealed the presence of maximum constituents in the chloroform extract, as it exhibited maximum numbers of well resolved spots. Despite showing maximum yield, the TLC profile of methanol extract was not much encouraging. The petroleum ether extract, on the other hand, despite showing very low yield exhibited quite interesting TLC profile, showing maximum number of resolved spots (Fig. 1-3). All of these TLC profiles may serve as characteristic fingerprint of *C. maxima* leaf. It would therefore be suitable for monitoring the identity and purity of the plant material and for detecting adulterations and substitutions.

The stable DPPH radical model is a widely used, relatively quick and precise method for the evaluation of free radical scavenging activity. The effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progressed, results in the scavenging of the radicals by hydrogen donation. It is visually noticeable that as a change in color from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidant activity of antioxidants (17). It has been reported that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect itself, forms the biological basis of chronic conditions such as arteriosclerosis (18).

Based on the data obtained from the present study, all the extracts were effective free radical inhibitor or scavenger that reacts with free radicals, which may limit free radical damage occurring in the human body. The results are summarized in the Table 1. A significant decrease in the concentration of DPPH radicals was due to the scavenging ability of the extracts and the reference compound. Free radical scavenging activity also increased with increasing concentration in the range of 2-15 µg/ml. The methanol extract was found to be the most potent even more than that of ascorbic acid at the test concentration, followed by the chloroform and petroleum ether extracts. The present preliminary study confirms marked *in vitro* free radical scavenging activity of *C. maxima* leaf which may be due to presence of multitude of constituents as revealed by TLC.

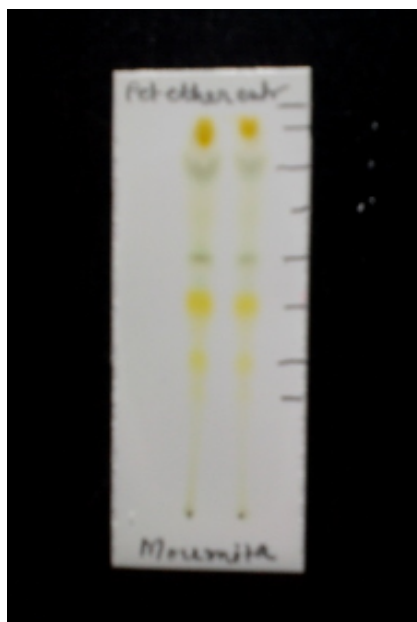


Fig. 1. TLC profile of the petroleum ether extract of *C. maxima* leaf. Solvent system: benzene: chloroform: ethyl acetate (5: 3: 2). R_f values: 0.40, 0.48, 0.60, 0.69, 0.80, 0.87, 0.95.

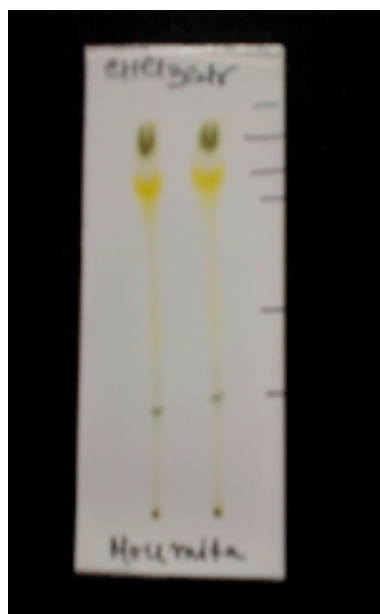


Fig. 2. TLC profile of the chloroform extract of *C. maxima* leaf. Solvent system: benzene: chloroform: ethyl acetate (2: 3: 5). R_f values: 0.30, 0.50, 0.77, 0.83, 0.92.

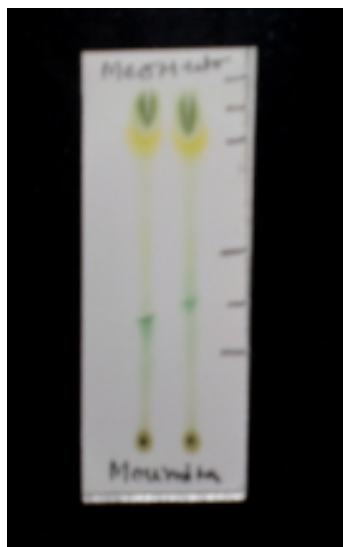


Fig. 3. TLC profile of the methanol extract of *C. maxima* leaf. Solvent system: chloroform: ethyl acetate (3: 7). R_f values: 0.26, 0.38, 0.52, 0.80, 0.90.

Table 1. DPPH scavenging effect of different extracts of *C. maxima* and ascorbic acid.

Extracts	Concentrations ($\mu\text{g/ml}$)	% of DPPH scavenging activity respectively	Mean \pm SEM
<i>C. maxima</i> (Petroleum ether)	2, 4, 6, 8, 10, 15	26.19, 31.27, 35.91, 58.51, 65.89 & 81.67	49.90 \pm 6.551
<i>C. maxima</i> (Chloroform)	2, 4, 6, 8, 10, 15	31.51, 38.62, 50.18, 58.92, 68.87 & 80.55	54.77 \pm 5.69
<i>C. maxima</i> (Methanol)	2, 4, 6, 8, 10, 15	47.79, 57.99, 71.35, 79.92, 88.74 & 98.81	74.10 \pm 5.17
Ascorbic acid	2, 4, 6, 8, 10, 15	25.70, 32.10, 42.50, 92.20 & 97.23	57.95 \pm 15.29

Acknowledgement

The authors are thankful to the authority of Bengal Institute of Pharmaceutical Sciences Kalyani, Nadia 741235, West Bengal, India for necessary facilities for the present study.

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