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Research article

Antioxidant study on *Cucurbita maxima fam Cucurbitaceae*

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Abstract

In any biochemical process there is biotransformation of one set of chemical substances to another. Classically, chemical reactions encompass changes that only involve the positions of electrons in the forming and breaking of chemical bonds between atoms, with no change to the nuclei (no change to the elements present).Antioxidant are substance An antioxidant is a molecule that inhibits the oxidation process. Here the present plant called *Cucurbita maxima* family Cucurbitaceae pericarp of fruit is selected for ita antioxidant study. The parameters which have studied are Gallic acid content, flavanols, flavanoids, nitric acid content, DPPH scavenging etc using gallic acid as standard. This article provides useful information regarding antioxidant property of plant *Curubita maxima* pericarp.

Keywords: Cucurbita maxima, flavanols, flavanoids.

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1. Introduction

Medicinal plants shows antioxidant property which are use to prevent oxidative damage caused by free radicals. Reactive oxygen species (ROS) are consisting of free radicals (O_2, HO) and non free radicals (H_2O_2). Free radicals produced from oxidation reaction start the chain reaction that damage the cell get involved in immune suppression, cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate the development of many diseases like cancer, liver injury, cardiovascular diseases, inflammation, diabetes, atherosclerosis etc . The most reactive free radical is the hydroxyl radical which is known to initiate lipid per oxidation and cause fragmentation of DNA leading to mutations. Naturally occurring antioxidants in leafy vegetables and seeds such as ascorbic acid, vitamin E and phenol compounds possess the ability to reduce the oxidative damage associated with many diseases. Cucurbita maxima Duch. Lam. (Cucurbitaceae) known as Dadhiphala in Sanskrit, Red Gourd in English and Kashiphala in Hindi, widely available throughout India. The chemical constituents from seeds contain 30% unsaturated fixed oil (linoleic and oleic fatty acids). Triterpenoids, flavonoids, coumarins, saponines, cucurbitacins, vitamins, minerals, notably zinc, amino acid known as cucurbitin which has the anthelminthic effect, high amount of carotenoid content which include lutein and beta-carotene. Therefore here we present investigation examining the antioxidant activity of Petroleum ether (PECM), Chloroform extract (CECM) and Methanolic extract (MECM) of pericarp of *C. maxima* (MECM) through various in vitro models.

2. Plant Materials and Extraction

Fruit of Cucurbita maxima (Cucurbitaceae) were collected from local market of Bhopal (M.P).The plant material was taxonomically identified by Botanical Survey of India (B.S.I), Pune, India. And a voucher specimen (No.SK-1) BSI/WC/Tech/2009/812 was retained in B.S.I. herbarium. The pericarp of *C. maxima* were scrapped, dried, powdered, sieved (60-80#) and successively extracted with petroleum ether (60-800), chloroform and methanol using Soxhlet apparatus.

Chemicals

Petroleum ether (60-800), Chloroform, Methanol, Gallic acid, Sodium carbonate, Folin-Ciocalteu reagent, stable 1,1-diphenyl-2-picrylhydrazyl (DPPH),ascorbic acid, Sulphanilamide ,Naphthylethylene diamine ,Sodium nitroprusside, Disodium hydrogen phosphate, Potassium dehydrogenate phosphate, Hydrogen peroxide, Ammonium molybdate, potassium iodide.

Equipment: UV- Visible spectrophotometer 2700 (Chemito) spectrum software 6.84

Photochemical evaluation of PECM, CECM and MECM [11]

500mg of the dried PECM, CECM and MECM was reconstituted in 10ml of petroleum ether, chloroform and methanol respectively and it was used for preliminary photochemical testing for the presence of different chemical groups of compounds.

Determination of total polyphenolic compounds [12, 13]

Total polyphenolic compounds were determined according to a protocol similar to that of Chandler and Dodds. MECM (50 µg/ml) was mixed with1 ml of Folin-Ciocalteu reagent (FCR) and incubated for 10 min. After that 4 ml 7.5% aqueous sodium carbonate solution was added. Tubes were vortexed and absorbance of blue colored mixtures recorded after 120 min at 740nm.The amount of total polyphenols was calculated as a Gallic acid equivalent from the calibration curve of Gallic acid standard solutions (concentration between10-100ug/ml) and expressed as percentage of Gallic acid equivalents (GAE) per gm of dry extract. Reading was taken against reagent blank.

Determination of total flavonoids [14, 15]

Aluminum chloride colorimetric method was used for flavonoids determination. One ml of MECM or standard solution of rutin (500µg/ml) was added to 10 ml volumetric flask containing 4 ml of H2O. To the above mixture, 0.3 ml of 5 % NaNO2 was added. After 5 min, 0.3 ml 10 % AlCl3 was added. At 6th min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with H2O. The solution was mixed well and the absorbance was measured against reagent blank at 510 nm. Total flavonoid content of methanolic extract was expressed as total mg rutin /g of extract. Formula: X = A. mo.10/ A0.m, Where, X=flavonoid content, mg/gm plant extract, A=the absorption of plant extract solution, A0=the absorption of standard rutin solution, m=the weight of plant extract, g; mo=the weight of rutin in solution in mg.

Determination of total flavonol [14, 15]

The content of flavonol was determined by Yermakov et al.(1987) The rutin calibration curve was prepared by mixing 2ml of 0.5,0.4,0.3,0.2,0.116,0.1,0.05,0.025 and 0.0166 mg/ml rutin ethanolic solution with 2 ml (2%w/w) aluminum trichloride and 6 ml (5%w/w) sodium acetate. The absorption at 440 nm was read after 2.5 hr at 20^0c.The sample procedure was carried out with 2 ml of plant extract (1%w/w) instead of rutin solutions. The content of flavonols, in rutin equivalents (RE) was calculated by the following formula: X= C.V/M, Where X= flavonol contents, mg/g plant extract in RE, C= the concentration of rutin solution established from calibration curve, mg/ml; V, M= the volume and the weight of plant extract in, ml, g.

DPPH radical scavenging activity [16, 17]

Free radical scavenging activity of extracts of pericarp of C. maxima was tested by its ability to bleach the stable 1, 1-diphenyl 2-picryl-hydrazyl (DPPH) radical. A stock solution of DPPH (0.3mM in methanol) was prepared such that 1ml of it in 3ml methanol gave an initial absorbance of 0.9.Decrease in absorbance in the presence of PECM, CECM and MECM at different concentration (50-500 mg/ml) were

noted after 15 min. scavenging activity was expressed as the %inhibition.

Formula:

% Inhibition =
Absorbance of the - Absorbance of the control test / Absorbance of the control
X100

Nitric oxide scavenging [18, 19]

Nitric Oxide radical inhibition estimated by the use of Griess Ilosvoy reaction (Garrat, 1964). The reaction mixture (3ml) containing sodium nitroprusside (10mM, 2ml), phosphate buffer saline (0.5ml) and different concentrations (50-300 mg/ml,0.5 ml) of PECM, CECM and MECM or standard solution (ascorbic acid ,10mg/ml, 0.5ml) was incubated at 25^{o}c for 150 min. After incubation, 0.5ml of the reaction mixture mixed with 1ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthylethylene diamine-dihydrochloride (0.1 % W/V) was added, mixed and allowed to stand for 30 min at 25^{0}c. A pink colored chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Ascorbic acid was used as a standard.

Hydrogen peroxide scavenging activity [20, 21]

A solution of hydrogen peroxide (40mmol L-1) was prepared in phosphate buffer (pH-7.4). Different concentrations (100-1500mg mL-1) of extracts (PECM,CECM and MECM) were added to the hydrogen peroxide solution (40mmol L-1,0.6 mL).Absorbance of hydrogen peroxide at 230nm was determined after 10 min against blank solution containing phosphate buffer without hydrogen peroxide. Percentage scavenging of hydrogen peroxide of the (PECM, CECM, and MECM) extracts and standard compound was calculated as per % inhibition formula.

3. Results and discussion

The extractive value of petroleum ether, chloroform and methanolic extracts were 9.95 %w/w, 2.25%w/w and 17.37%w/w respectively. Preliminary phytochemical screening of PECM, CECM, MECM showed the presence of steroid/ terpenoids, high amount of phenolic, glycosides, tannins and flavonoids (table-1).

Further antioxidant study was done using different parameters such as total polyphenolic content, total flavanol content, total flavanoid content, DPPH scavenging, Nitric oxide scavenging (Table-2.1, 2.2, 2 and table 3).

Subsequent quantification of phenolics of MECM and other extract was done by Folin-Ciocalteau method which showed the presence of good amount of total phenolics (12mg/gm calculated as per gallic acid) total flavonoid and flavonol content was found to be 3.8 mg/gm and 0.8 mg/gm (rutin equivalent) of MECM respectively. IC-50 value for DPPH method of PECM, CECM and MECM were found to be 393ug/ml, 355ug/ml and 155ug/ml (Graph-1) 155ug/ml, while for nitric oxide scavenging method 280ug/ml, 303ug/ml and 211ug/ml were found closes to standard refrence of gallic acid (Graph-2) and for Percentage scavenging of hydrogen peroxide of the (PECM, CECM, and MECM) extracts and standard compound was calculated as per % inhibition formula which was MECM shows its acitivity at 545ug/ml, 273ug/ml and 619ug/ml was found to be close to (Graph-3) respectively. The activity was due to the presence of phytoconstituents in extract. From this study it further proves that pericarp of *Cucrbita maxima* (MECM) shows more potent antioxidant activity as compared to PECM and CECM.

Table-2: Total phenolic content of MECM

Content	Absorbance +,-SD(n=3)	Mg/gm
Total polyphenolic content a	0.305± 0.052 12	12

Table 2.1: Total flavanoid content of MECM

Total flavonoid content	b 0.112 ±0.006 0.38	0.38
Total flavonol content c	0.327±0.004 0.08	0.08

Table 2.2: Total flavanol content of PECM, CECM and MECM:
Values represented ±SD (n=3)

S. N	Chemical group	PECM	CECM	MECM
1	Carbohydrates	absent	absent	present
2	Steriods/ Terpenoids	present	absent	absent
3	Flavanoids	absent	absent	present
4	Alkaloids	absent	absent	absent
5	Tannins	absent	absent	present
6	Phenols	absent	absent	present
7	Glycoside	absent	absent	present

a = expressed as mg of gallic acid equivalent/ gram of dry plant extract

b = expressed as mg of rutin equivalent/ gram of dry plant extract

c = expressed as mg of rutin equivalent of dry plant extract

Table 3: DPPH free radical Scavenging activity of Cucurbita maxima (MECM) at different concentration

Conc (microgram /ml)	absorbance	% inhibition	absorbance	% inhibition	absorbance	% inhibition
10	0.856±0.070	7.55	0.920±0.020	9.13	0.868±0.038	5.24
50	0.79±0.010	14.68	0.836±0.021	21.95	0.730±0.010	20.3
100	0.733±0.045	20.96	0.718±0.009	25.76	0.591±0.053	43.34
150	0.675±0.039	27.1	0.683±0.012	32.17	0.433±0.056	52.72
300	0.452±0.008	51.18	0.624±0.025	40.2	0.417±0.064	54.47
500	0.39±0.005	57.88	0.478±0.029	48	0.289±0.007	68.4

Control**=**0.926±0.021

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