International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.2, pp 1533-1538, April-June 2010

In vitro Antioxidant activity of Pericarp of *Cucurbita maxima* Duch. ex Lam

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Abstract: *Cucurbita maxima* Duch. ex Lam.(Cucurbitaceae)known as *Dadhiphala in Sanskrit,* contains triterpenoids ,flavonoids, coumarins, saponines, cucurbitacins, carotenoid, vitamins, minerals and amino acid. The present study was undertaken to evaluate in vitro antioxidant activities of petroleum ether, chloroform and methanolic extract of pericarp of *C.maxima* .Total polyphenolic, flavonoid and flavonol content of MECM found12mg /gm (gallic acid equivalent),3.8 mg/gm and 0.8 mg/gm (rutin equivalent) respectively.All extract were tested for DPPH radical scavenging, Nitric oxide radical scavenging and Hydrogen peroxide scavenging activity.IC-50 value for DPPH method of PECM,CECM and MECM were found to be 393ug/ml, 355ug/ml and 155ug/ml , while for nitric oxide scavenging activity 280ug/ml, 303ug/ml and 211ug/ml and for hydrogen peroxide scavenging activity 545ug/ml, 273ug/ml and 619ug/ml respectively..MECM shows good antioxidant activity as compare to PECM and CECM.
Key words: *Cucurbita maxima*; total polyphenolic; total flavonoids; DPPH; Nitric oxide; Hydrogen Peroxide.

Introduction

Medicinal plants show antioxidant property are used to prevent oxidative damage caused by free radicals.[1] 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[2,3]. The most reactive free radical is the hydroxyl radical which is known to initiate lipid peroxidation and cause fragmentation of DNA leading to mutations.[4]

Although the body possesses such defense mechanisms as enzymes and antioxidant nutrients, which arrest the damaging properties by removing free radical intermediates and inhibit other oxidation reactions.

Naturally occurring antioxidants in leafy vegetables and seeds such as ascorbic acid, vitamin E and phenolic compounds possesses the ability to reduce the oxidative damage associated with many diseases[5,6].

Cucurbita maxima Duch. ex Lam.(Cucurbitaceae)known as *Dadhiphala in Sanskrit, Red Gourd in English and Kashiphala in Hindi* , widely available in temperate regions such as North America and Australia. 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. It has also been suggested that phytosterols present in the seed may play some role in the treatment of prostate problem.[7, 8] Long chain hydrocarbons and fatty acids in fruits, spinasterol in flowers have been reported.Pulp is applied to burns,scalds, inflammations, abscesses ,boils and is remedy for migraine ,neuralgia, haemoptysis & hemorrhages [9,10]

Therefore we undertook the present investigation to examine 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.

Plant Materials and Extraction

Fruit of *Cucurbita maxima* (Cucurbitaceae) were collected from local market of Nasik (Maharashtra).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/2008/712 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(60-80^0),chloroform and methanol using Soxhlet apparatus.

Chemicals:

Petroleum ether (60-80^0), Chloroform, Methanol, Gallic acid, Sodium carbonate, Folin-Ciocalteu reagent, stable 1,1-diphenyl-2-picrylhydrazyl (DPPH),ascorbic acid, Sulphanilamide ,Naphthyl ethylene diamine ,Sodium nitroprusside, Disodium hydrogen phosphate, Potassium dihydrogen phosphate, Hydrogen peroxide, Ammonium molybdate ,potassium iodide.

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

Phytochemical 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 phytochemical 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 H_2O. To the above mixture, 0.3 ml of 5 % $NaNO_2$ was added.. After 5 min, 0.3 ml 10 % $AlCl_3$ was added. At 6th min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with H_2O. 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:$X = A.\ m_o.10/\ A_0.m$,Where, X=flavonoid content, mg/gm plant extract, A=the absorption of plant extract solution, A_0=the absorption of standard rutin solution, m=the weight of plant extract, g; m_o =the weight of rutin in solution in mg.

Determination of total flavonol[14,15]

The content of flavonol was determined by Yermakov, etal.(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^{0}c.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 were 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 µg/ml) were noted after 15 min. scavenging activity was expressed as the %inhibition.

Formula **:** % Inhibition =

$$\frac{\text{Absorbance of the control} - \text{Absorbance of the test}}{\text{Absorbance of the control}} \times 100$$

Nitric oxide scavenging [18,19]

Nitric Oxide radical inhibition estimated by the use of Griess Illosvoy reaction (Garrat, 1964). The reaction mixture (3ml) containing sodium nitroprusside (10mM, 2ml), phosphate buffer saline (0.5ml) and different concentrations (50-300 µg/ml,0.5 ml) of

PECM, CECM and MECM or standard solution (ascorbic acid ,10µg/ml, 0.5ml) was incubated at 25°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 naphthyl ethylene diamine dihydrochloride (0.1 % W/V) was added, mixed and allowed to stand for 30 min at 25°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 (100-1500µg mL^{-1}) of extracts (PECM,CECM and MECM) were added to the hydrogen peroxide solution (40mmol L^{-1},0.6mL).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 .

Result 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).Subsequent quantification of phenolics in MECM by Folin-Ciocalteau method showed the presence of good amount of total phenolics(12mg/gm calculated as per gallic acid),total flavonoid and flavonol content found to be 3.8 mg/gm and 0.8 mg/gm (rutin equivalent) 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), while for nitric oxide scavenging method 280ug/ml, 303ug/ml and 211ug/ml (Graph-2) and for hydrogen peroxide scavenging activity 545ug/ml,273ug/ml and 619ug/ml (Graph-3) respectively. The activity may be due to the presence of phytoconstituents in extract.MECM is more potent in antioxidant activity as compare to PECM and CECM.

Table-1: Preliminary phytochemical screening of PECM, CECM and MCM

Sir. no.	Chemical groups	PECM	CECM	MECM
1	Carbohydrates	--	--	+++
2	Steroids/ Terpenoids	+++	--	---
3	Flavonoids	--	--	+++
4	Alkaloids	--	--	--
5	Tannins	--	--	+++
6	Phenols	--	--	+++
7	Glycoside	--	--	++

Table-2: Total phenolic, flavonoid and flavonol content of MECM

	Absorbance±SD (n=3)	**Mg/gm**
Total polyphenolic content [a]	0.305± 0.052	12
Total flavonoid content [b]	0.112 ±0.006	0.38
Total flavonol content [c]	0.327±0.004	0.08

Values represented ±SD (n=3)

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

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

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

Table -3: DPPH free radical Scavenging activity of *Cucurbita maxima*.

	PECM		**CECM**		**MECM**	
Conc.(µg/ml)	Absorbance ±SD (n=3)	% Inhibition	Absorbance ±SD (n=3)	% Inhibition	Absorbance ±SD (n=3)	% Inhibition
Control	0.946±0.036	--	0.0949±0.028	--	0.948±0.007	--
10	0.841±0.006	11.09	0.852±0.018	11.38	0.633±0.039	33.22
50	0.782±0.005	17.33	0.795±0.080	16.22	0.536±0.002	0.43.25
100	0.725±0.008	23.36	0.72±0.098	24.13	0.466±0.001	50.84
150	0.677±0.018	28.43	0.630±0.139	33.61	0.400±0.001	57.80
200	0.631±0.010	33.29	0.602±0132.	36.56	0.293±0.010	69.09
300	0.567±0.018	40.06	0.467±0.042	50.79	0.257±0.003	72.89
500	0.0448±0.045	52.64	0.400±0.020	57.85	0.714±0.002	81.64
IC_{50}	-	**393µg/ml**		**355µg/ml**		**155µg/ml**
IC_{50} (Std.) Ascorbic acid			**16µg/ml**			

SD= Standard deviation, PECM= Pet. Ether Extract of *C.maxima*, CECM= Chloroform Extract of *C.maxima* , MECM= Methanol Extract of *C.maxima*.

Table -4: Nitric oxide radical Scavenging activity of *Cucurbita maxima*

	PECM		**CECM**		**MECM**	
Conc.(µg/ml)	Absorbance ±SD (n=3)	% Inhibition	Absorbance ±SD (n=3)	% Inhibition	Absorbance ±SD (n=3)	% Inhibition
Control	0.926±0.021	--	0.920±0.020	--	0.916±0.036	--
10	0.856±0.070	7.55	0.836±0.021	9.13	0.868±0.038	5.24
50	0.79±0.010	14.68	0.718±0.009	21.95	0.730±0.010	20.3
100	0.733±0.045	20.96	0.683±0.012	25.76	0.591±0.053	43.34
150	0.675±0.039	27.1	0.624±0.025	32.17	0.433±0.056	52.72
200	0.452±0.008	51.18	0.55±0.001	40.2	0.417±0.064	54.47
300	0.39±0.005	57.88	0.478±0.029	48	0.289±0.007	68.4
IC_{50}	-	**280µg/ml**		**304µg/ml**		**211µg/ml**
IC_{50} (Std.) Ascorbic acid			**258µg/ml**			

SD= Standard deviation, PECM= Pet. Ether Extract of *C.maxima*, CECM= Chloroform Extract of *C.maxima* , MECM= Methanol Extract of *C.maxima*.

Table-5:Hydrogen peroxide scavenging activity of *C. maxima*

	PECM		**CECM**		**MECM**	
Conc.(µg/ml)	Absorbance ±SD (n=3)	% Inhibition	Absorbance ±SD (n=3)	% Inhibition	Absorbance ±SD (n=3)	% Inhibition
Control	0.931±0.015	0.00	0.937±0.040	0.00	0.956±0.016	0.00
100	0.701±0.146	24.70	0.569±0.075	39.27	0.776±0.052	18.82
200	0.613±0.177	34.50	0.372±0.061	60.29	0.638±0.127	33.26
300	0.526±0.211	43.50	0.270±0.076	71.18	0.515±0.157	46.12
500	0.319±0.127	65.73	0.202±0.018	78.44	0.399±0.173	58.26
1000	0.194±0.063	79.16	0.144±0.020	84.63	0.263±0.077	72.48
1500	0.087±0.033	90.65	0.085±0.511	90.92	0.151±0.043	84.20
IC_{50}	-	**545µg/ml**		**273µg/ml**		**619µg/ml**
IC_{50} (Std.) Ascorbic acid			**405µg/ml**			

SD= Standard deviation, PECM= Pet. Ether Extract of *C.maxima*, CECM= Chloroform Extract of *C.maxima* , MECM= Methanol Extract of *C.maxima*.

Figure no.1: DPPH free radical Scavenging activity of *Cucurbita maxima*.

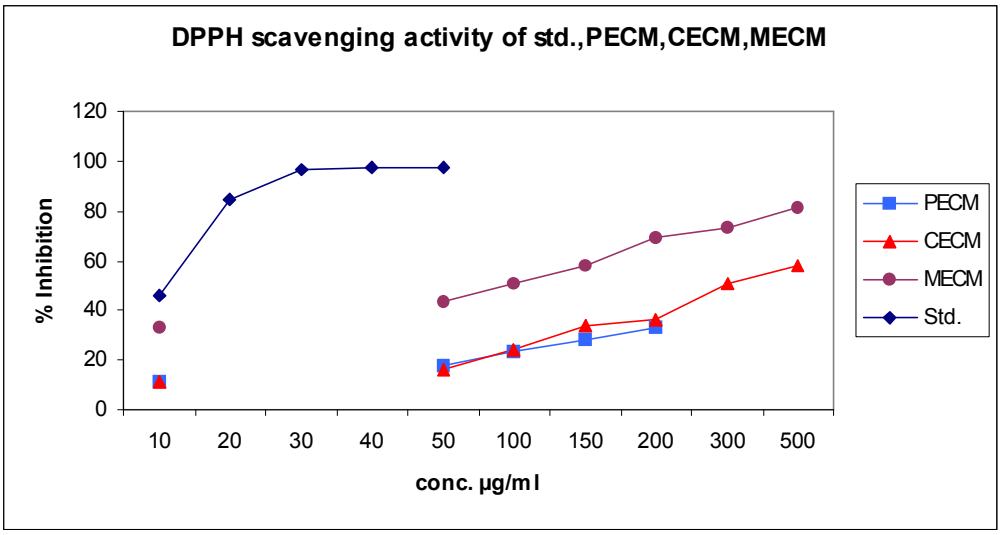


Figure no.2:Nitric oxide radical Scavenging activity of *Cucurbita maxima*

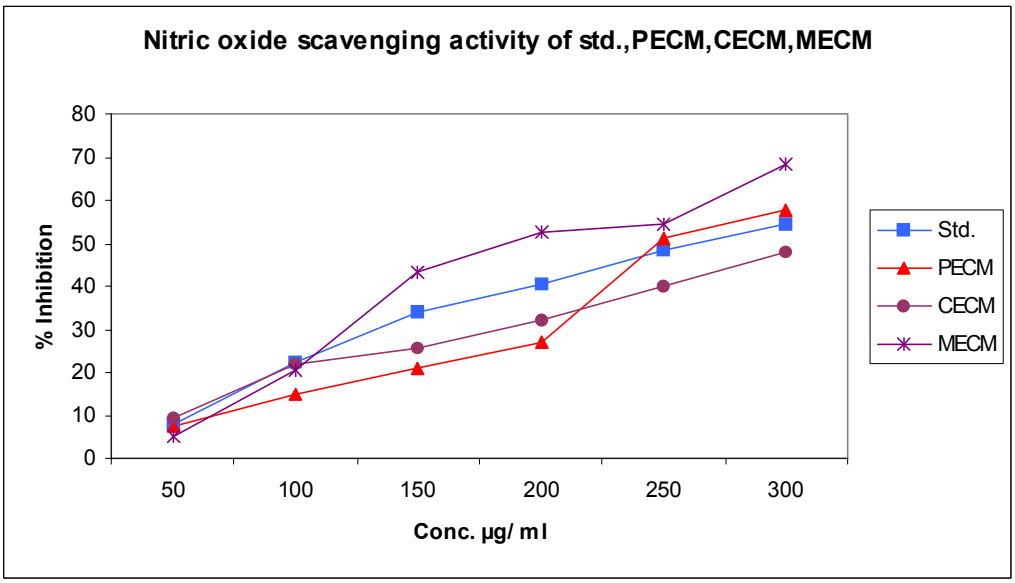
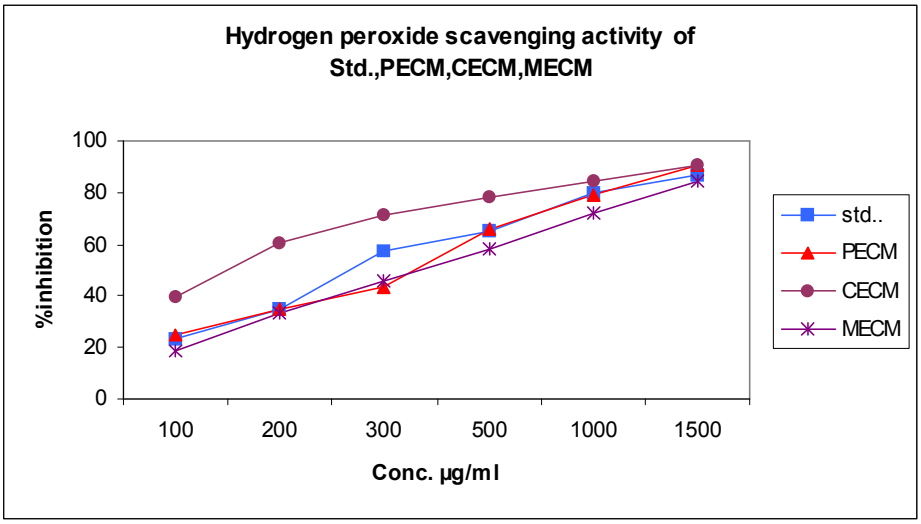


Figure no.3: Hydrogen peroxide scavenging activity of *C. maxima*



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