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TOTAL PHENOLIC CONTENT AND ANTIOXIDANT POTENTIAL OF CUCURBITA MAXIMA (PUMPKIN) POWDER

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ABSTRACT: As Antioxidant potential of many plants is mainly due to phenolic components such as phenolic acid, phenolic diterpense therefore total phenolic content and antioxidant potential of cucurbita maxima. (Pumpkin) powder was determined respectively by using Folin-Ciocalteu reagent and diphenyl-picryl-hydrazyl (DPPH) assay. In this method the antioxidants present in the plant extracts reacted with DPPH, which is a stable free radical and converted it to 1,1-diphenyl-1,2-picryl, hydrazine. Total phenolic content of pumpkin was determined using Folin-Ciocalteu reagent and gallic acid as standard, amount of phenolic compound was found to be 6.5 mg/gm powder of the drug. The high fiber content of pumpkin helps to aid proper digestion and in traditional medicine it is used as an anthelmintic, taenicide, diuretics and in the treatment of hemorrhoids so we compared the anti oxidant potential of pumpkin powder with marketed preparation (Pilex tablet). The half maximal inhibitory concentration (IC₅₀) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. IC₅₀ value of pilex tablet = 8.53 µg/ml & IC₅₀ value of pumpkin powder =9.12 µg/ml.

INTRODUCTION: A *Cucurbita maximum* is an annual climber plant found in most places. It is in flower from Jul to September, and the seeds ripen from Aug. to October. Pumpkin is common name for *Cucurbita maxima* it is commonly used as vegetable. Pumpkin has been much used as a medicine in Central and North America.

It is a gentle and safe remedy for a number of complaints, especially as an effective tapeworm remover for children and pregnant women for whom stronger acting and toxic remedies are unsuitable⁴. The high fiber content of pumpkin helps to aid proper digestion.

Pumpkin seeds have the most powerful therapeutic effect. They help to eliminate intestinal parasites, stave blood vessels, regulate cholesterol, and stimulate kidney activity.

Pumpkin seeds are of great help, through their energizing role in the treatment of cancer, leukemia, multiple sclerosis or various difficult to cure diseases.

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Pumpkin seed has been used in traditional medicine as an anthelmintic (an agent used to expel intestinal worms), taeniocide (an agent which kills tapeworms) and as a diuretics^{2,3}.

Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.⁴ As Antioxidant potential of many plants is mainly due to phenolic components such as phenolic acid, phenolic diterpene therefore total phenolic content and antioxidant potential of *Cucurbita maxima* (pumpkin) powder was determined using DPPH (1, 1-diphenyl-2-picryl-hydrazyl) is stable free radical and methanolic solution of it is used to evaluate the anti-oxidant activity of the several natural compounds. The phenolic compound presents in plants possess many hydroxyl groups which have very strong radical scavenging activity. Antioxidant on interaction with DPPH, either 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 drug¹⁰. The change in the absorbance produced at 517 nm, has been used as a measure of antioxidant activity⁶. In traditional medicine pumpkin was used as taeniocide, diuretics, to aid the proper digestion and also high fibre content of pumpkin used as stool softener because of this pumpkin also used in the treatment of hemorrhoids. Therefore the antioxidant potential of pumpkin powder was determined in comparison with marketed preparation of Himalaya Herbal Health Care i.e. Pilex Tablet used in the treatment of hemorrhoids.

MATERIALS AND METHOD:

Plant Materials & Chemicals: *Cucurbita maxima* (pumpkin) family cucurbitaceae was collected in the month of August – September from local area of Kopargaon, Maharashtra, India. The authentication of the plant was done in the Department of botany, SSGM College of Kopargaon, Dist. Ahmednagar Tal. Kopargaon, Maharashtra, India. Then pulp of pumpkin fruit was collected and dried in the sunlight and

powdered in the mixer and then pass through sieve no 120.

Chemicals and reagents: DPPH, Galic acid, Folin Ciocalteu's reagent and ascorbic acid was obtained from Store department of Sanjivani College of pharmacy, kopargaon, all chemicals are of AR grade.

Estimation of Total Phenolic compounds: Total phenolics were determined using Folin-Ciocalteu reagent and gallic acid as standard. Gallic acid solution was prepared by dissolving 10 mg of gallic acid in 100 ml distilled water in volumetric flask (100µ/ml stock solution) From above stock solution 0.5 to 2.5 ml aliquots were pipette out into 25 ml volumetric flasks. Ten ml of distilled water and 1.5 ml of Folin-Ciocalteu's reagent were added to each above volumetric flask. After 5 min, 4 ml of 20% sodium carbonate solution was added and the volume was made up to 25 ml with distilled water and incubated at room temperature for 30 min and the absorbance of solution was recorded at 765nm and standard curve of absorbance verses concentration of gallic acid was plotted⁵.

1gm of pumpkin powder treated or extracted with 70 % methanol (15×3 times), filtered, pooled and the volume was adjusted to 50 ml with 70 % methanol in a volumetric flask. From stock solution suitable quantity of extract was taken in to a 25 ml volumetric flask and 10 ml of water and 1.5 ml of Folin-Ciocalteu's reagent were added to it. The mixture was kept for 5 min and then 4 ml of 20 % sodium carbonate solution was added and made up to 25 ml with double distilled water. The mixture was incubated at room temperature for 30 min and the absorbance was recorded at 765 nm in a spectrometer^{5, 7}. Percentage of total phenolics was calculated from calibration curve of gallic acid (50 – 250 µg/ml) plotted using the above procedure and total phenolics were expressed as % gallic acid.

Determination of Antioxidant activity: The percentage radical scavenging activity was determined according to method given by DPPH (1, 1-diphenyl-2-picryl-hydrazyl) is stable free radical and methanolic solution of it is used to evaluate the anti-oxidant activity of the several natural compounds.

Antioxidant potential of pumpkin was determined using DPPH and ascorbic acid as standard. Pumpkin powder extract and pilex tablet extract was used as test solution.

Reagent:

DPPH solution – (%w/v): It was prepared by dissolving 33 mg of DPPH in one liter of analytical grade methanol and kept in amber coloured bottle to protect from sunlight.

Sample preparation: In sample preparation 1 gm of pumpkin powder was taken and dissolved in 50 ml distilled water, sonicate the sample for 20 min then centrifuged and collect the residue of powder, weighed it and calculate the amount of powder dissolved. Prepared the dilution of different concentration 100 – 1000 µg/ml of stock solution was prepared. Similarly for pilex tablet, initially weight of tablet was taken then remove the coating of tablet, weighed the powder of tablet and dissolved in distilled water. Similarly calculate amount of powder dissolved and prepared the stock solution of 100-1000µg/ml.

Ascorbic acid solution preparations (% w/v): 100 µg/ml stock solutions were prepared by dissolving 10 mg of ascorbic acid 100ml with distilled water from this 10, 20, 30, 40, 60, and 80µg/ml.

Procedure: 1 ml of different concentration of extract (test solution) solution and standard were taken in different vials. To this 5 ml of methanolic solution of DPPH was added, shaken well and mixture was incubated at 37°C for 20 min. Measure the absorbance against methanol as blank at 517 nm. Take absorbance of the DPPH as control, Percent antiradical activity can be calculated by using following formula¹⁰;

% Antiradical activity=

$$\frac{\text{Control Abs} - \text{sample A}}{\text{Control Abs}} \times 100$$

IC₅₀ value was determined from plotted graph of % inhibition vs different concentration of test solution.

RESULT:

Total phenolic content: The total phenolic compounds estimation from pumpkin powder showed the absorbance of 1.214 at 765 nm wavelength and phenolic contents was found out to be 6.5 mg/gm powder of the drug. The total phenolic content in the powder of pumpkin was determined by using Folin-Ciocalteu's method. The sample extract dilution was oxidized with Folin-Ciocalteu reagent and the absorbance of the resulting blue color was measured at 765 nm after 30 min. The result of Folin-Ciocalteu's method revealed the presence of total phenolic contents 6.5 mg/gm of powder which was not reported earlier. Phenolic constituents are very important in the plant because of their scavenging ability due to their hydroxyl groups. Phenolic compounds are famous powerful chain breaking antioxidants and has been reported that phenolic compounds are associated with antioxidant activity. From graph, concentration of extract equivalent to gallic acid is 5.2 µg /ml, therefore total phenolic content of pumpkin powder is 6.5 mg/gm powder.

TABLE 1: STANDARD (GALLIC ACID)

Sr. no.	Gallic acid concentration	Absorbance (765)
1.	0.5	0.2438
2.	1	0.4862
3.	1.5	0.7118
4.	2	0.9640
5.	2.5	1.1649

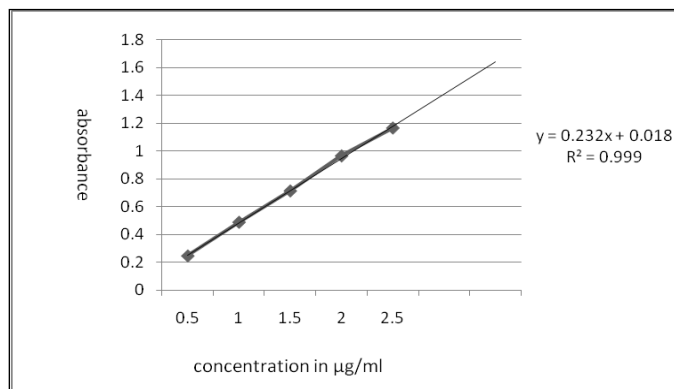


FIGURE 1: GRAPH OF TOTAL PHENOL CONTENT

DPPH Radical Scavenging Activity: DPPH is stable nitrogen centered free radical which can be effectively scavenged by antioxidants and shows strong absorbance at 517 nm.

The change in absorbance of DPPH radical caused by the extracts was due to the reaction between the antioxidant molecules and the extracts, which resulted in the scavenging of the radical by hydrogen donation. It was visually noticeable as a discoloration from purple to yellow. Extent of DPPH radical scavenged was determined by the decrease in intensity of violet color in the form of IC_{50} values⁷.

The absorbance of control was found to be **1.5438**. From graphs IC_{50} value of pilex tablet and pumpkin powder was calculated, The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process.

TABLE 2: % INHIBITION OF PILEX TABLET & % INHIBITION OF PUMPKIN POWDER

Sr. no	Conc. (ppm)	Absorbance at 517nm	% Inhibition of Pilex tablet	Absorbance at 517 nm	% inhibition of Pumpkin powder
1	100	1.0554	33.65	1.0857	29.67
2	200	0.9852	38.06	1.0513	31.90
3	300	0.9655	39.30	1.0100	34.57
4	400	0.9353	41.20	0.9622	37.67
5	500	0.9251	41.84	0.9135	40.82
6	600	0.8582	46.05	0.8790	43.06
7	700	0.8215	48.35	0.8488	45.01
8	800	0.8258	48.08	0.8258	46.50
9	900	0.7925	50.94	0.7898	48.84
10	1000	0.7582	52.30	0.7341	52.44

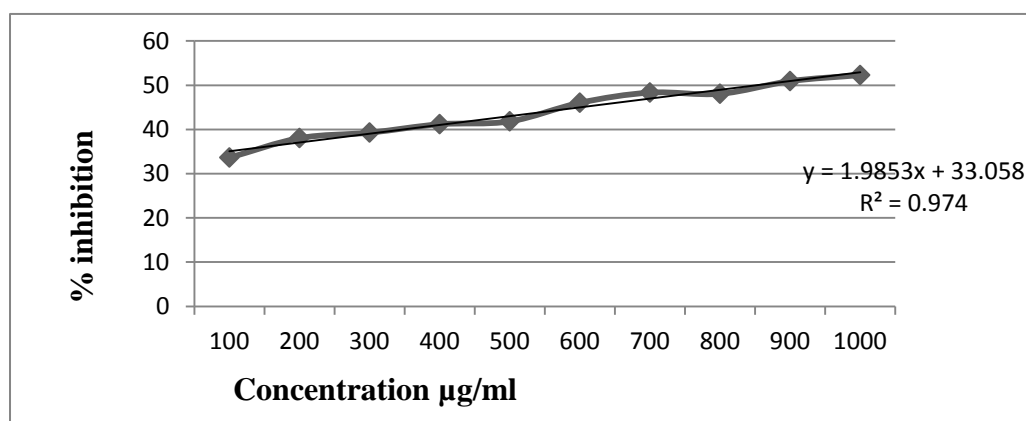


FIGURE 2: GRAPH OF PILEX TABLET –CONCENTR

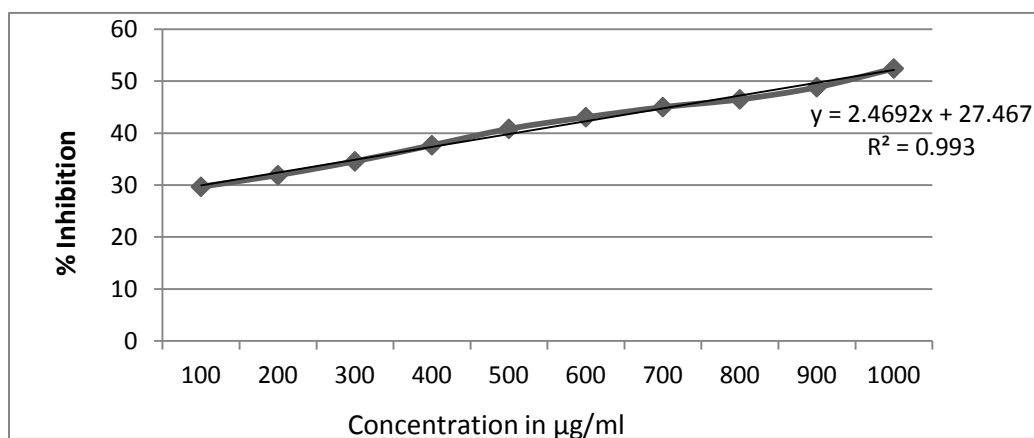


FIGURE 3 : GRAPH OF PUMPKIN POWDER – CONCENTRATION VS % INHIBITION

IC_{50} value of pilex tablet = 8.53 μ g/ml; IC_{50} value of pumpkin powder =9.12 μ g/ml

DISCUSSION AND CONCLUSION: The present study suggest that the powder of cucurbita maxima (pumpkin) shows presence of phenolic compounds. These phenolic compounds posses many hydroxyl group including o – hydroxy groups which have very strong antioxidant potential. It also include comparison between antioxidant potential of pumpkin powder with pilex tablet.

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