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# PRELIMINARY ANALYSIS OF PUMPKIN SEED EXTRACT FOR PHYTOCHEMICAL, ANTIOXIDANT ABILITY, CYTO-TOXICITY AND COLLAGEN SYNTHESIS

**Akashraj R. Agrahari[1], Pranjali P. Dhawal[2] and Dr. Tanuja V. Tirodkar[3*]**

[1,3]Department Of Biotechnology, KET's V.G. Vaze College Of Arts, Science And Commerce Mulund East, Mumbai-40008, India.
[2]Animal Biotechnology And Biochemistry Divison, KET's Scientific Research Centre, Vaze College Campus, Mithagar Road, Mulund East, Mumbai- 400 081, India.

***Corresponding Author: Dr. Tanuja V. Tirodkar**
Department Of Biotechnology, KET's V.G. Vaze College Of Arts, Science And Commerce Mulund East, Mumbai-40008, India.

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## ABSTRACT

Pumpkin seed extracts are greatly studied and have gained importance due to its nutritional benefits. The present study aims to explore other properties beneficial to mankind. The crude extract of pumpkin seed was obtained by cold extraction method. This crude extract was further analyzed for anti-oxidant activity using 2,2-Diphenyl picryl hydrazyl method (DPPH method) . The Phytochemicals present in the seed extract were found by the preliminary methods. Cyto-toxicity analysis on 3T3/NIH Cell line was carried using MTT assay. The Collagen synthesis activity of the crude extract was also tested on 3T3 cell line. After testing all the parameters of the Pumpkin seed crude extract, it was found that it had DPPH radical scavenging ability. The extract when tested for toxic effects showed that even at concentration 1 mg/mlit had proliferative ability as compared to cell with media and without extract. The extract also boosted the Collagen synthesis by 3.91 pg/ml as compared to media control. The properties exhibited by Pumpkin seed crude extract could be recommended in Pharmacological and Cosmetological applications with proper clinical trials.

**KEYWORDS:** Pumpkin seed crude extract, Anti-oxidant, DPPH, Collagen synthesis, Non-toxic.

## INTRODUCTION

Pumpkin has been cultivated in Mexico and North America since at least 14000 BC based on archaeological evidence.[1] Pumpkin seeds are known to be good source of lipids, potassium, manganese,etc.[2] Pumpkin seeds are also noted to be useful in cure of benign prostatic hyperplasia(BPH) in animal models like rats.[3] Pumpkins are annual climbers and are grown in almost all the parts of India. Pumpkin seeds are also good source of iron which helps in proper regulation and maintenance of menstrual cycles in women.[4]

These all various properties of Pumpkin seeds makes Pumpkin seeds one of the super foods that must a part of everyone's diet. Despite all these known properties of Pumpkin seeds, there is not much reported on the cosmetological properties of these seeds. In the current research conducted, the prime focus is on the finding some novel applications of pumpkin seed extracts in skin care products.

## METHODS AND MATERIALS

### Materials

2,2 diphenyl picrylhydrazyl was procured from Himedia, Mumbai, India. MyBioSource Col-4 ELISA Kit was procured from Krishgen, Mumbai. Dulbecco's Modified Eagle's Media DMEM was purchased from Himedia, Mumbai, India, Fetal Bovine Serum, FBS was purchased from Gibco, Thermo Fischer, USA, IU/mL Penicillin and 100µg/mL Streptomycin was purchased from Himedia, Mumbai, India. All the other chemicals used were of analytical grade

### Plant Material

The pumpkin seeds were collected from the local vegetable market. These seeds were then once soaked in water and dried at room temperature for 48hours .Further they were kept at 45 to 50 degrees Celsius for one day to ensure that they lose all the moisture. The germinated seeds were removed manually to ensure uniformity of the sample.These seeds were grinded fine using a mixer grinder and this powder was used for the further extraction processes.

**Methods**

**Cold Extraction**

10grams of powder was weighed and were added into a conical flask. To this conical flask, 100 ml of ethanol was added. This conical flasks was kept on Rotary Shaker at 120 rpm at room temperature for 24 hours.The solution inside the flask was then filtered using Whatmann Filter paper number 1. The filtrate was collected in the evaporating dish. The evaporating dish containing this filtrate was kept in the incubator at 50$^{o}$C until the solvent in the dish got evaporated and left behind was the crude extract. This extract is then used for carrying out the further tests and assays.

**Detection of Phytochemicals**

The phytochemicals of the ethanolic extract were assessd using the below standard procedures-

**Test for flavonoids:** To the test solution add few drops of sodium hydroxide solution, intense yellow color is formed on addition of few drops of dilute acid which indicates the presence of flavonoids.[5]

**Test for tannins:** Treat the extract with ferric chloride solution, blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.[5]

**Test for cardiac glycosides:** 5ml of the extract sample is dissolved in 5ml of water. Add 2ml. glacial acetic acid with 3ml ferric chloride solution; further add 6 drops Sulphuric acid. A brown ring will be observed in the solution which indicates the presence of cardiac glycosides.[5]

**Test for saponins:** 2ml. solution of extract is taken in a test tube with water and was shaken well vigorously. Production of foam in the upper layer of the solution indicates the presence of saponins.[5]

**Test for steroids:** 5ml extract solution was mixed with few ml of chloroform and shaken with concentrated sulphuric acid. After standing for some time it yields red color.[6]

**Test for alkaloids:** Alkaloids give reddish brown precipitate with Dragendroff's reagent.[5]

**Test for Terpenoids**: 2.0ml of chloroform was added with the 5 ml extract and evaporated on the water path and then boiled with 3 ml of $H_2SO_4$ concentrated. Formation of grey color shows the presence of terpenoids.[5]

**Test for Phenols:** 1mL of extract was dissolved in 2mL of distilled water. Into this solution, a few drops of 5% ferric chloride solution were added. The formation of dark green color indicated the presence of phenolics compounds.[6]

**Test for Phlobatannins:** 1mL of extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate indicated the presence of phlobatannins.[5]

**Antioxidant Activity**

The antioxidant activity of the extract was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay.[7,8] Different concentrations of seed extract was prepared from respective solvent ranging from 10µl/ml to 100µl/ml using methanol as a diluent. 500µl of this extract was mixed with 500µl of DPPH solution of concentration $10^{-6}$M. The seed extract (500µl seed extract + 500µl methanol) was used as a blank. Negative control was prepared by adding 500µl methanol and 500µl DPPH. Ascorbic acid was used as a positive control. The tubes were incubated in dark for 20 minutes. The absorbance of the mixture was then measured at 517 nm using a spectrophotometer.

The extent of its activity can be checked with the formula,

Percent Scavenging Activity = C-T/C *100

Where, C=Negative Control and T=Test.

**Cytotoxicity on 3T3 fibroblast cell line**

**Maintenance of Cell lines**

Mouse Embryo Fibroblast cell line (NIH/3T3) was taken to check the Cytotoxicity of ethanolic extract of *Cucurbita moschata* seeds using MTT Assay. The cells were maintained at 37°C/5%$CO_2$ in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS. The media was changed thrice a week and sub-cultured twice.

**MTT Assay**

The cells were trypsinized and seeded in a 96 well plate at a cell density of 0.5 x 10 4 cells/ml in 100µL of media per well. The plate was incubated at 37°C/5% $CO_2$ for 24 hours. The media was removed and 100µL of plant extract of different concentrations made in media. The plate was incubated for 24 hours. After 24 hours of incubation, the media was removed and 10µL of 5mg/ml of MTT was added to each well. The plate was incubated in dark for 3 hours. The formazan formed was extracted using 100µL of DMSO. The plate was read at 530 nm after 30 min. The experiment was conducted in triplicates.[9]

The percent viability was calculated by the below-mentioned formula,

Percentage Viability = (Absorbance of test/ Absorbance of media control) x100
Media control represents 100% cells.

**Estimation of Collagen**

Cells were seeded in at a cell density of $0.5x10^4$ cells/ml in 100µL of media per well in a 96 well plate. Ethanolic extract was added at a concentration of 1 mg/ml. After 24 hours the increase in collagen content was estimated using the instructions given the kit. This ELISA kit is designed for the quantitative determination of Collagen Type IV.

## RESULTS AND CONCLUSIONS

**Phytochemical Assay**

The ethanolic extract of pumpkin seed was tested for the presence of various phytochemicals (Table.1).

**Table 1: Phytochemicals present in Seed Extract.**

| PHYTOCHEMICALS TESTED | RESULT |
|---|---|
| Flavonoids | Present |
| Tannins | Present |
| Cardiac Glycosides | Present |
| Saponins | Present |
| Steroids | Present |
| Alkaloids | Present |
| Terpenoids | Present |
| Phenols | Present |
| Phlobatannins | Present |

**Antioxidant Activity**

Ethanolic extract of pumpkin was used for the determination of its antioxidant property. Following is the result obtained in the form of a graph. 100µl of pumpkin seed liquid extract could scavenge the free radical by 17.28%. (Fig1.).

Further study can also be done on the dried extract of pumpkin seeds in order to increase the antioxidant potential of subsequent extract. The effect of different extraction procedure, drying of extract, effect of various solvents and subsequent effect in anti-oxidant ability should be studied. The extract was dried for cytotoxicity and collagen measurements.

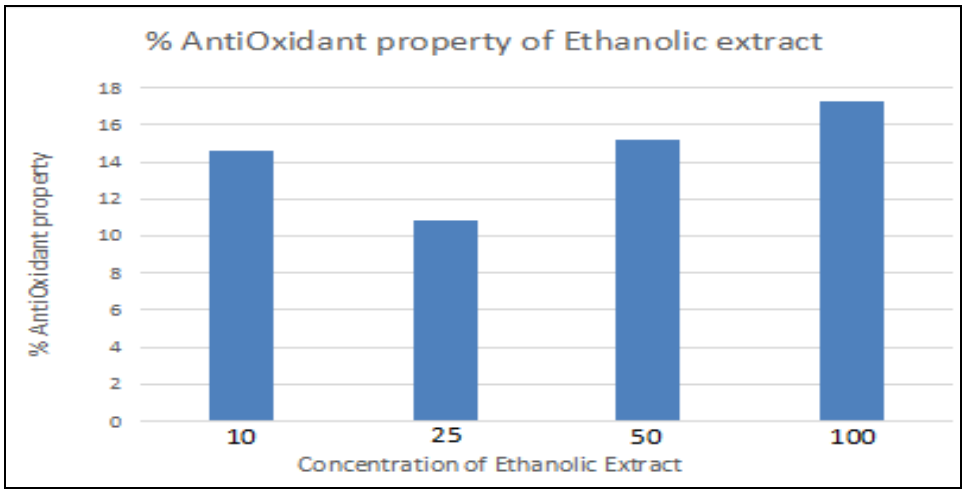


**Figure 1: DPPH radical scavenging ability of Pumpkin seed extract.**

**Cytotoxicity on 3T3 fibroblast cell line**

Even at a higher concentration as 1000µg/ml of pumpkin seed extract the no cell death was observed. In fact an increase in cell growth as compared to media control was seen that explains the proliferative effect of pumpkin seed extract on fibroblast cell line. It was observed that the ethanol extract of the *Cucurbita moschata* seed extract was Nontoxic to the cells. The results for cytotoxicity of the seed extract are given below-(*Table-2)*

**Table 2: Proliferative ability of Pumpkin seed extract.**

| Sample | % Viability | % Proliferation |
|---|---|---|
| Ethanolic extract of *Cucurbitamoschata* Seeds | 115.0975 | 15.1 |
| Media Control | 100 | 0 |

**Estimation of Collagen**

The ethanolic extract of pumpkin seeds promoted the collagen synthesis as compared to media with 10% FBS.From the standard curve for collagen 4 from 1.5- 24 pg/ml (Fig.2) of collagen present in the standard collagen, the amount of collagen synthesized by the

proliferative effect of pumpkin seeds was estimated. It was found that the collagen content in the wells containing pumpkin seed extract showed increased levels of Collagen by 3.91 pg/ml as compared to media control.

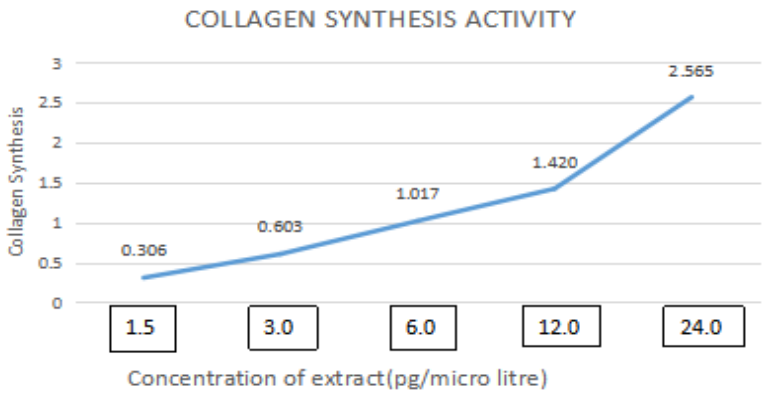


**Figure 2: Standard Curve Readings for Collagen Synthesis Activity**

## DISCUSSION

Antioxidant property for the pumpkin seed extract was evaluated using DPPH assay. In one study, β-carotene bleaching by linoleic acid assay was conducted for testing the antioxidant property of the pumpkin seeds. They compared their results with the standard antioxidant i.e. BHT, which gave comparative results. [10] The toxicity study on 3T3 Cell line revealed that the extract under study had proliferative properties. The collagen synthesis activity of the seed extract was also carried out that showed increase in concentration of collagen. Based on these results we can opt for a number of applications. In literature, pumpkin seed oil has been tested for wound healing property in rats. It was found that the hydroxyproline which is used as a biomedical marker for collagen was more in the test group of rats which were treated with the pumpkin seed oil. That group of rats showed better and faster recovery from the wounds as compared to the standard reference drug that was used i.e. Cicaflora cream.[10] Similar results were obtained with respect to collagen synthesis and proliferative ability in the current study.

## CONCLUSION

TheEthanolic extract of *Cucurbitamoschata* seeds showed the presence of phytochemicals which had implications in Antioxidant activity against DPPH free radical. The extract also showed proliferative effect on the growth of fibroblast cells *in vitro*. The extract also showed increased collagen content in comparison to media control. The pumpkin seed extract therefore has implications in wound healing, can reduce ageing, and related cosmetic abilities.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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