



Phytochemical Screening and Evaluation of Protein content in the Seed extracts of *Cucurbita maxima*

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Abstract

Nature has been a source of medicinal agents since time immemorial. *Cucurbita maxima* seeds have many health benefits as they are a good source of protein, zinc, and other vitamins. The objective of this study was to identify the compounds in the *Cucurbita maxima* seed extracts. The crude ethanol and chloroform extracts of *Cucurbita maxima* were subjected to preliminary screening. The Phytochemical screening of the *Cucurbita maxima* seed extracts revealed the presence of polyphenols, alkaloids, flavonoids, terpenoids, and glycosides. The chemical screening further showed the presence of various amino acids. The seed homogenate of *Cucurbita maxima* showed the presence of reasonable quantity of proteins.

Key-Words: *Cucurbita maxima*, Phytochemical, Protein, Homogenate

Introduction

Herbal medicine is the study and use of medicinal properties of plants. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans and can be used to effectively treat human disease. At least 12,000 such compounds have been isolated so far. Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs. Thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines^{1,2}.

India is the largest producer of medicinal herbs and a rich heritage of traditional medicine constituting with its different components like ayurveda, siddha, unani, homoeopathy and naturopathy^{3, 4}. The genus *cucurbita* indigenous to the western hemisphere is comprised of five domesticated Species⁵.

Cucurbita maxima is one of the most diverse domesticated species perhaps with more cultivated forms than any other crop⁶. This species originated in South America from the wild *Cucurbita maxima* sp⁷. Different squash types of this species were introduced in to North America as early as the 16th century.

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Secondary centers of diversity include India, Bangladesh and Burma. The most important pharmacological activities of *Cucurbita maxima* are purgative activity, cytotoxic, antitumor, hepatoprotective, anti-inflammatory and fertility⁸.

Scientific classification

Kingdom : Plantae
Division : Magnoliophyta
Order : Violales
Family : Cucurbitaceae
Genus : *Cucurbita*
Species : *maxima*
Binomial Name: *Cucurbita maxima*

Botanical description of *Cucurbita maxima*

Pumpkin is the common name for the genus *Cucurbita* of the family Cucurbitaceae (gourd family), a group that includes the pumpkins and squashes. The pumpkin varies much in form, being sometimes nearly globular, but more generally oblong or ovoid in shape. The rind is smooth and very variable in colour. The seed is cooling and of the nature of the Melon. An annual creeper with stems up to 30 feet (9 m) long, furnished with large clasps. The leaves are large and rough like Melons. The flowers are large like yellow Lilies in colour. The fruit is very large and contains white flattish seeds.

Medicinal uses of *Cucurbita maxima*

The seed of *Cucurbita maxima* has pharmacological activities such as anti bacterial, anti- inflammatory and antioxidant effects. The most critical health benefit attributed to *Cucurbita maxima* seed oil is preventing the growth and reducing the size of the prostate⁹.

Cucurbita maxima seed can be used as antitumor, immunomodulatory and antibacterial agent.

Proteins in *Cucurbita maxima*

Cucurbitacin is a major seed globulin in this plant. It is described as a 11s class globulin storage protein weighing 325,000D. It has also two non identical but very similar major subunit (alpha and beta) each weighing approximately 54, 000D from the storage globulin as alpha3 and beta 3 hexamer¹⁰.

In view of this, the present study was undertaken with the aim of studying phytoconstituents, amino acids and protein content of *Cucurbita maxima* seed extract.



Cucurbita maxima *Cucurbita maxima* seed

Material and Methods

Plant source

The fresh seeds of *Cucurbita maxima* were purchased from a supermarket in Kanchipuram district. The plant seeds were ground to a uniform powder using a milling machine. The powder was kept in an airtight container for further use.

Chemicals Used

Bovine serum albumin, copper sulphate, sodium carbonate, sodium potassium tartarate, Folin's phenol Reagent etc. were obtained from the standard chemical companies like Qualigens and SRL.

Preparation of Solvent Extracts

Ethanol Extraction

5g of powdered seed was used separately for the preparation of extract. Sample was packed between folds of filter paper and placed in soxhelet apparatus, run between 60-80°C using Ethanol as solvent.

Chloroform Extraction

5g of powdered seed was used separately for the preparation of extract. Sample was packed between folds of filter paper and placed in soxhelet apparatus, run between 60-80°C using chloroform as solvent.

Phytochemical screening

Chemical tests were carried out on the ethanol and chloroform extracts of the powdered seed using standard procedures to identify the constituents as described by Harborne (1998)¹¹.

1. Test for alkaloids

a) Mayer's test

To a few ml of filtrate, a few drops of Mayer's reagent was added by the side of the tube. A creamy white precipitate indicates the presence of alkaloids.

2. Test For flavanoids

a) Shinoda's test

To 5ml of the extract, 5-10 drops of dilute HCl and small piece of magnesium chloride was added and the solution was boiled for a few minutes. Appearance of reddish pink colour or dirty brown colour indicates the presence of flavanoids.

3. Test for carbohydrates

a) Benedict's Test

To 0.5ml of the filtrate, 0.5ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic red colour precipitate indicates the presence of sugar.

4. Test for saponins

a) Froath test

To 0.05ml of filtrate, added 5ml of distilled water and shaken vigorously for a stable persistence froath. Froathing which persisted on warming indicates the presence of saponins

5. Test for tannins

a) Ferric Chloride test

To 2ml of extract, few drops of 5% ferric chloride solution was added. The appearance of violet colour indicates the presence of tannins.

6. Test for phytosterols

a) Libermann-Burchard's test

To 2ml of the filtrate, 2ml of acetic anhydride was added. Concentrated sulphuric acid was added along the sides of the test tube. A colour change from violet to blue indicates the presence of phytosterols.

7. Test for terpenoids

a) Salkowski Test

5ml of the extract was mixed with 2ml of chloroform and concentrated sulphuric acid was added to form a layer. A reddish brown colour indicates the presence of terpenoids.

8. Test for proteins**a) Ninhydrin Test**

To 1ml of extract few drops of Ninhydrin reagent was added and heated in a boiling water bath. A purple blue colour indicates the presence of proteins

b) Biuret Test

To 1ml of extract, equal volume of 5% NaOH solution and copper sulphate solution added. A blue colour indicates the presence of proteins.

9. Test for anthraquinone

To 5ml of extract, added dilute H_2SO_4 and 1ml of diluted ammonia. Appearance of pink colour indicates presence of anthraquinone

10. Test for polyphenols

10ml of plant extract was heated for 30 minutes in a water bath. 1ml of 1% $FeCl_3$ was added to the mixture followed by the addition of 1% potassium ferricyanide. The mixture was filtered and formation of green-blue colour indicates the presence of polyphenols.

11. Test for glycosides**a) Borntrage's test**

To 0.5ml of extract, few drops of HCl was added and heated in a boiling water bath for few minutes and treated with chloroform. The chloroform layer was separated and added equal volume of diluted ammonia. Appearance of pink colour indicates the presence of glycosides.

Phytochemical analysis of amino acids**Sample preparation**

The solid part left after soxhlet extraction was digested with 6N HCl in boiling water for half an hour and used for amino acid analysis.

1. Ninhydrin test

To 1ml of sample, add 5drops of Ninhydrin Reagent. Heated in a boiling water bath for 2 min. A purple colour indicates the presence of aminoacids.

2. Xanthoproteic test

To 3ml of sample, add 1ml of concentrated nitric acid and heated for 3min. Then cooled and added 0.5 ml of NaOH. Reddish orange colour indicates the presence of aromatic amino acids.

3. Folin's test

To 1ml of sample, add 1ml of Folin's phenol reagent followed by the addition of 1N sodium carbonate. Blue colour indicates presence of tyrosine and tryptophan.

4. Millon's test

To 1ml of sample, add 1ml of millon's reagent and heated for 3 minutes. Then 1% sodium nitrate is added. Red colour formed indicates the presence of Tyrosine.

5. Pauly's test

To 1ml of sample, add 1ml of 1% sulphanilic acid and cooled in ice. Then 1ml of sodium nitrite added. After

5 min, 2ml sodium carbonate added. Presence of cherry red colour indicates the presence of Histidine.

6. Morner's test

To 1ml of sample, add 2ml of Morner's reagent and heated for 3 minutes. Green colour indicates presence of Tyrosine.

7. Hopkin's Cole test

To 1ml of sample, add equal volume of glyoxalic acid followed by the addition of concentrated sulphuric acid along the sides of the test tube. A violet colour ring formed at the junction of two layers indicates the presence of tryptophan.

9. Ehrlich's test

To 1ml of sample, 1ml of Ehrlich's reagent added. Blue colour indicates presence of Tryptophan.

10. Sakaguchi's test

To 1ml of sample, add 5 drops of NaOH and 4 drops of α -naphthol. Shaken well. To this 10ml of bromine water was added. Red colour indicates Arginine.

11. Sodium nitroprusside test

To 1ml of sample, add 1.5ml of NaOH, 0.5ml of sodium nitroprusside and 1.5ml of 1% glycine. Boiled for 1min and cooled. Add 3ml of 6NHCl and allow stand for 15min. Reddish purple colour indicates the presence of Methionine.

12. Sodium plumbate test

To 1ml of sample, add equal volume of 45% NaOH. The contents are boiled for 2min and cooled. Then add 0.5ml of lead acetate. Dirty black colour indicates the presence of cysteine.

Biochemical analyses**Tissue Preparation for biochemical analyses**

100 mg of the plant tissue was weighed, uniformly homogenized with 1.0 ml of 0.5 M phosphate buffer, pH 6.9. The homogenate was centrifuged and the supernatant was used for the following biochemical assays.

Estimation of protein

The total protein content was estimated by the method of Lowry et al. (1951)¹². To 0.1 ml of sample, 4.5 ml of alkaline copper reagent was added. The mixture was shaken well and allowed to stand for 20 min. Then, 0.5 ml of Folin's phenol reagent was added, mixed well and incubated at 37°C, for 15 min. Standards containing BSA at concentrations from 20 μ g to 100 μ g were treated similarly. The blue color developed was read at 620 nm in a spectrophotometer. The total protein content was expressed in mg/g of tissue.

Estimation of Albumin

The albumin was estimated by Dye - binding method of Doumas B.T et al., (1971)¹³. 0.02ml of sample was made to 3ml with saline (.9% NaCl). Then 4ml of

Bromocresol green dye solution was added and mixed well. The blue colour developed was read at 620nm in colorimeter. The albumin content was expressed as mg/g of tissue.

Estimation of Globulin

The amount of globulin was determined by subtracting the albumin content from the total protein. The globulin content was expressed as mg/g of tissue.

Determination of A/G ratio

A/G ratio is arrived by dividing the albumin content by globulin content.

Results and Discussion

Table 1 and 2 show the phytochemical analysis of ethanol and chloroform extract of the *Cucurbita maxima*. Most of the phytochemicals present in both the organic solvent extracts were identical. It reveals the presence of medicinally active constituents such as alkaloids, flavanoids, saponins, tannins, polyphenol, carbohydrates, phytosterol, glycosides, proteins, terpenoids, phenols and Anthraquinones. Many of these plant materials have been investigated for novel drugs for the production of new therapeutic agents. Plants produce about 7000 different pharmaceutically important compounds and a number of top selling drugs of present time are helpful in treating many diseases¹⁴.

Recent observations have shown that changes in cell-wall polysaccharides profiles in various fruits and vegetables are related to change in firmness and texture during ripening, storage, and processing, suggesting that these polysaccharides play important roles in the plants¹⁵⁻¹⁶. *Cucurbita maxima* seed have many health benefits, as they are a good source of protein, zinc, and other vitamins, and they are even said to lower cholesterol. *Cucurbita maxima* seed has been claimed to combat benign prostatic hyperplasia. *Cucurbita maxima* seed oil contains essential fatty acid that help maintain healthy blood vessels, nerves and tissues.

Nandave et al.,¹⁷ reported the cardioprotective property of flavanoids. It possesses potential pharmacological activities such as antioxidant activity, vitamin C sparing activity, and the activities of 5-lipoxygenase and cyclo-oxygenase. Flavanoids have free radical scavenging and antioxidation properties, which are useful for their pharmacological activities including anticancer and anti-ageing properties. Flavanoids show interactions with cytochrome p-450, antileukemic properties, and mild vasodilators properties useful for the treatment of heart disease¹⁸.

The data on amino acids profile of the *Cucurbita maxima* seed proteins are presented in Table 3. El-Adawy and Taha¹⁹ also observed the abundance of

these amino acids in pumpkin seed flour. The earlier studies reported that pumpkin seed was superior to soybean in its content of all amino acids except lysine, which is in agreement with our findings. The fairly high concentration and the wide spectrum of the amino acids detected in the *Cucurbita maxima* whole seed make them suitable for fortification of foods.

It has been reported that histidine plays an important role to treat rheumatoid arthritis²⁰. *Cucurbita maxima* seeds are greater reservoir of amino acid and are rated among the highest organic source including tyrosine. It has been reported that tyrosine promotes breaking down of environmental toxin and helps our body to fight with depression²¹.

Protein contents of *Cucurbita maxima* seed are presented in Table 4. The pumpkin seeds contain a high percentage of crude protein. Storage protein of pumpkin seed is 11s type globulin comprising more than 80% of the protein content in the dry seeds. Pumpkin globulin has an acidic polypeptide (mol wt, 34,000) and basic polypeptide (mol wt, 22, 000) linked by disulfide bond²². Similar values for protein content of the pumpkin seeds were reported by Evangelos S. Lazos²³. The crude protein value compared favorably with high protein seeds and legumes like soybeans and cowpea. However, it is higher than others such as lima beans and chickpeas²⁴.

Pumpkin generally contains large amounts of starch, free sugars, and vitamin such as B1, B2, C, as well as several active ingredients, including β -carotene and γ -amino butyric acid²⁵. A major component of plant seeds is frequently a globulin, highly enriched in gln, asn, and arg, which is thought to serve primarily as a nitrogen reserve. It has been reported that a large proportion of the dry weight of the mature seeds is protein²⁶.

Table 1: Analysis of the phytochemicals in ethanol and chloroform extracts of *Cucurbita maxima* seed

S/No.	Phytochemicals	Ethanol	Chloroform
1.	Alkaloids	+	+
2.	Flavanoids	+	+
3.	Carbohydrates	+	+
4.	Glycosides	-	+
5.	Polyphenols	+	+
6.	Tannins	+	+
7.	Phenols	+	+
8.	Phytosterols	-	-
9.	Saponins	+	+
10.	Terpenoids	+	+
11.	Proteins	+	+
12.	Anthraquinones	-	-

Table 2: Analysis of the amino acids in ethanol and chloroform extracts of *Cucurbita maxima* seed

Amino acid	Ethanol	Chloroform
Ninhydrin	+	+
Xanthoproteic	+	+
Folin's	+	+
Millon's	+	+
Pauly's	-	+
Morner's	+	+
Hopkin's cole	+	+
Ehrlich's	+	-
Sakaguchi's	-	-
Sodium plumbate	-	-
Sodium nitroprusside	-	-

Table 3: The levels of proteins in the *Cucurbita maxima* Seed tissue

Parameters	Values
Protein (mg/gm of tissue)	100
Albumin (mg/gm of tissue)	30
Globulin (mg/gm of tissue)	70
A/G ratio	0.428

Conclusion

The current study shows that *Cucurbita maxima* contain many important phytochemicals. This plant seed plays very important role in the field of medicine. The investigations on *Cucurbita maxima* seed extract for the phytochemicals and active ingredients could provide leads to interesting pharmaceuticals of plant origin. The high protein of the *Cucurbita maxima* seed coupled with a fairly high concentration of the amino acids make it suitable for fortification of foods.

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