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Qualitative phytochemical screening of various extracts of *Cucurbita maxima* seeds

Hukma Ram Parihar, Ashok Gaur, Kartar Singh, Pratishtha Sharma and Aruna Panwar

Abstract
Cucurbita maxima commonly known as pumpkin, belongs to the family Cucurbitaceae and is widely cultivated throughout the world for use as vegetable as well as medicine. The present study deals with qualitative phytochemical evaluation of aqueous and alcoholic extracts of *Cucurbita maxima* seeds. The study includes preparation of various extracts by successive solvent extraction for phytochemical analysis. Dried seeds of pumpkin were taken and then grinded to make coarse powder of it, later its aqueous and alcoholic extracts prepared in Soxhlet apparatus. Qualitative phytochemical screening of various extracts of *Cucurbita maxima* seeds reveals the presence of alkaloids, reducing sugars, glycosides, tannins.

Keywords: *Cucurbita maxima*, seeds, phytochemical and soxhlet apparatus

Introduction
Cucurbita maxima (family: Cucurbitaceae) commonly known as "Pitakusmandah" in Sanskrit; "Kaddu" or "Petha" in Hindi and "Pumpkin" in English. It is widely cultivated throughout India and in most warm regions of the world, for use as vegetable as well as medicine. Both of its fruits and the aerial parts are commonly consumed as vegetable (Ambasta, 1992) [3]. The fruits are sweet, having various medicinal properties *viz.* refrigerant, emollient, diuretic, sedative and tonic, so they are useful in burns, scalds, inflammations, abscesses, boils, migraine and neuralgia (Prajapti, 2006) [7]. The seeds of *Cucurbita maxima* used as anthelmintic, diuretic and nervine in various disease conditions. Seeds are also used as abortifacient and insecticidal (Ambasta, 1992; Prajapti, 2006) [3, 7]. This plant has been traditionally used in many countries such as India, China, Brazil, Yugoslavia and America as antidiabetic and antihyperlipidemic (Sharma, 2013) [9], antitumor, antihypertensive, anti-inflammatory, immunomodulatory and antibacterial agents (Adolfo, 2005; Agarwal, 1991; Ambasta, 1992; Jia, 2003; Popovic, 1971; Prajapti, 2006) [1, 2, 3, 4, 6, 7]. The present study is designed to explore the preliminary phytochemical evaluation of *Cucurbita maxima* seeds, which is responsible for its pharmacological activities.

Materials and Methods
Collection and processing of plant material: The dried mature seeds *Cucurbita maxima* were procured from local market and coarse ground for preparation of extracts.

Preparation of extract
The seeds were cleaned well with water and dried in a shadow place. After complete drying, the seeds were powdered and were extracted by using soxhlet apparatus, successively with water and alcohol. Then solvent was immediately separated by using a rotary vacuum evaporator assembly. The obtained semi solid mass was further dried in tray drier machine. All the extracts obtained were stored in screw capped air tight vials under cold place for qualitative analysis and pharmacological studies.

Phytochemical analysis
The phytochemical analysis was undertaken to determine the presence of various active constituents of aqueous and alcoholic extracts of *Cucurbita maxima* seeds by conducting the various tests.

1. Test for alkaloids
A little extract was taken in 5 ml of 1.5 % hydrochloric acid (v/v) and filtered through Whatman's filter paper No. 1. The filtrate was used for testing alkaloids.

A. Dragendorff's test
Dragendorff's reagent: It was prepared by mixing solution A (1.7 g of bismuth subnitrate + 20 g of tartaric acid + 80 ml of distilled water) and solution B (16 g potassium iodide + 40 ml of distilled water) in equal (1:1) proportion (v/v) and allowed to stand for a few minutes. From this solution a working standard was prepared by taking 10 ml of this solution and adding 20g of tartaric acid and making its volume up to 100 ml with distilled water.

Procedure: The working reagent was sprayed on a filter paper and the paper was dried. The sample solution was applied on the paper using a capillary tube. Development of an orange-red colour indicates the presence of alkaloids.

B. Wagner's test
Wagner's reagent: 1.27 g iodine and 2.0 g potassium iodide were dissolved in distilled water and the total volume was made to 100 ml for preparing Wagner's reagent.

Procedure: Take 5ml of acid solution of the extract and then added 2ml of Wagner's reagent in it. Appearance of brown flocculent precipitate indicates the presence of alkaloids.

2. Test for reducing sugars
A. Benedict's test: About 5 ml of dissolved extract was taken with equal quantity of Benedict's reagent and heated. The appearance of brownish red precipitate (reduction) was indicative to presence of reducing sugars.

B. Fehling's test: Two ml of aqueous solution of extract in a test tube was added into 5 ml of Fehling's reagent (mixture of equal volumes of Fehling's solutions A and B) and boiled in a water bath for about 2 min. The brick-red precipitate was indicative of the presence of reducing sugars.

3. Test for glycosides
A. Benedict's reagent test: The solution obtained in Benedict's test for reducing sugars was filtered and 1 ml dilute hydrochloric acid was added to it for hydrolyzing the glycosides. Equal quantity of Benedict's solution was added to it and boiled in hot water bath. Appearance of brownish precipitate suggested the presence of glycosides.

B. Fehling's reagent test: This test is performed with the solution obtained in Fehling's test. To the clear solution a few drops of dilute hydrochloric acid was added and boiled for 5 minutes for hydrolyzing glycosides. Fehling's reagent was again added to note any further reduction, which indicates the presence of glycosides.

4. Test for sterols
A. Salkowski reaction: 0.5 g of extract was dissolved in 2 ml concentrated chloroform in a test tube. Then 2ml of concentrated sulphuric acid was added to it by the side wall of test tube slowly drop by drop. Development of red colour in the chloroform layer and greenish florescence in the lower part of solution are suggestive of the presence of sterols in extracts.

B. Lieberman Buchard reaction: 0.5 g of extract was dissolved in 2 ml chloroform in a test tube. 5-10 drops of acetic anhydride were added followed by addition of 0.5 ml concentrated sulphuric acid by the side wall of the test tube. Transient colour development from red to blue and finally green colour indicates the presence of sterols.

5. Test for proteins
A. Xanthoprotein test: One gram of extract was taken in a test tube containing 2 ml distilled water and 0.5 ml concentrated nitric acid was added to it. Appearance of a yellow precipitate suggests the presence of protein.

B. Biuret test: One gram of extract was taken in tube having 1 ml distilled water. Then 1 ml of 1 per cent sodium hydroxide solution was added to it. To this, 1-2 drops of 1 per cent copper sulphate solution were added. Development of violet colour indicates the presence of protein.

6. Test for tannins: One gram of extract was taken with 5 ml of methanol and warmed and then filtered. The filtrate was divided into two parts and tested with following reagents.

A. Lead acetate test: A few drops of lead acetate solution were added to the methanolic extract. The formation of precipitate indicates the presence of tannins.

B. Ferric chloride test: A few drops of ferric chloride solution were added to the little of the above filtrate. Development of green colour in the filtrate indicates the presence of tannins.

7. Test for flavonoids
A. Alkaline reagent test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

B. Lead acetate test: Extract was treated with few drops of lead acetate solution. Formation of a yellow coloured precipitate indicates the presence of Flavonoids.

8. Test for saponins
A. Foam test: One gram of extract was taken in a test tube containing 5 ml of sodium bicarbonate and 5 ml of water and, the contents were shaken vigorously. Formation of a stable froth suggests the presence of saponins.

9. Test for anthraquinones
A. Bontrager's Test: 0.5g of extract was boiled in a test tube for 1-2 minutes with 5 ml of 10 per cent sulphuric acid and filtered immediately. The filtrate was cooled and shaken with benzene. The benzene layer was separated and the same was shaken with half of its volume of 10 per cent ammonia. Development of pink ring in ammonical layer indicates the presence of anthraquinones.

Results and Discussion
Aqueous and alcoholic extracts of *Cucurbita maxima* seeds gave the presence of following phytochemicals are presented in Table 1.

Table 1: Qualitative phytochemical analysis of aqueous and alcoholic extracts of *Cucurbita maxima* seeds.

Active principle	Test applied	Aqueous extract of *Cucurbita maxima* seeds	Alcoholic extract of *Cucurbita maxima* seeds
Alkaloids	Dragendorff's reagent	+	+
	Wagner's reagent	+	+
Reducing sugars	Benedict's reagent	+	+
	Fehling's reagent	+	+
Glycosides	Benedict's reagent	+	-
	Fehling's reagent	+	-
Sterols	Salkowski reaction	-	-
	Lieberman Buchard reaction	-	-
Proteins	Xanthoprotein test	-	-
	Biuret test	-	-
Tannins	Lead acetate test	+	+
	Ferric chloride test	+	+
Flavonoids	Alkaline reagent test	-	-
	Lead acetate test	-	-
Saponins	Foam test	-	-
Anthraquinones	Bontrager's test	-	-

Key: + = Present; - = Absent

In the present study, aqueous extract of *Cucurbita maxima* seeds revealed the presence of alkaloids, reducing sugars, glycosides, tannins. Muchirah *et al*. (2018) [5] reported the presence of various active principles like alkaloids, carbohydrates, tannins, glycosides and terpenoids on phytochemical screening of aqueous extract of *Cucurbita maxima* seeds. Alcoholic extract *Cucurbita maxima* seeds revealed the presence of alkaloids, reducing sugars, tannins. Ravishankar *et al*. (2012) [8] reported the presence of carbohydrates, steroids, proteins and amino acids in ethanolic extract of seeds of *Cucurbita maxima*. The phytochemical composition of ethanolic extract of *Cucurbita maxima* seeds revealed the presence of various active principles like alkaloids, carbohydrates, tannins and terpenoids (Muchirah *et al*., 2018) [5]. Sharma *et al*. (2013) [10] conducted the phytochemical evaluation of extract of *Cucurbita maxima* seeds and reported the presence of proteins, carbohydrates, flavonoids, saponins and tannins.

Conclusion

In the present study, phytochemical analysis of aqueous and alcoholic extract of *Cucurbita maxima* seeds revealed the presence of alkaloids, reducing sugars, tannins. Glycosides were present in aqueous extract of *Cucurbita maxima* seeds only. The phytochemical constituents of *Cucurbita maxima* seeds may have several medicinal properties and can be utilized for the treatment of various diseases. So it reveals that pumpkin is an important annual herb with various therapeutic applications.

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