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STANDARDIZATION AND COMPARATIVE PHYTOCHEMICAL SCREENING OF *CUCURBITA MAXIMA'S* DIFFERENT EXTRACTS OBTAINED BY DIFFERENT EXTRACTION TECHNIQUES

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ABSTRACT

Extraction is the separation of medically active portions of plant tissue using a selective solvents through standard procedure. For extraction purpose Pumkin seed powder was used which containing alkaloids, glycosides, & steroids. General technique of extractions were used maceration, sonication, hot continuous extraction (soxhlet) by a different solvents i.e., water, methanol, acetone and n-hexane. In this Soxhlet extraction technique was given the highest % yield of extract as compared to sonication and maceration extraction method. By performing phytochemical screening we concluded that the alkaloids, glycosides and steroids were present in the extract and also performing crude drug standardization. The plant should be authenticated by Y.C. Institute of Science, Satara.

KEYWORD: Extraction, maceration, sonication, hot continuous extraction**.**

INTRODUCTION

Extraction as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures.[1] The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use.

A **pumpkin** is a cultivar of a squash plant, most commonly of *Cucurbita pepo* that is round, with smooth, slightly ribbed skin, and deep yellow to orange coloration. The thick shell contains the seeds and pulp.[2] *Cucurbita maxima* are one of the cultivated squash species native to south America. These pumpkins are also cultivated in India's Bangladesh and Burma. The vine of cucurbita maxima can be easily differentiated by its growing stalk which

gets swollen and croky when the fruit is ripe.[3] It contains moisture, lipids, protein and ash. Non-polar lipids, glycosides and phospholipids. The major fatty acid present in the lipids was linolenic.[4] Popularity of Cucurbita maxima (pumpkin) in various traditional system of medicine for several ailments focused the investigator's attention on this plant. The present study is designed to explore the preliminary physicochemical and phytochemical evaluation of *Cucurbita maxima* seeds, which is responsible for its pharmacological activities.[5] Pumpkin seeds, seed oil, and pumpkin pulp have been evaluated in limited clinical trials for medicinal actions, including anthelmintic, hypotensive, and hypoglycemic activity.[6] The extracts may also be useful for managing symptoms of benign prostatic hyperplasia and anxiety-related disorders, although limited clinical trial information is available. There are various methods of extraction maceration, percolation, decoction and ultrasonication.[7] Amongst the various methods of extraction were selected to prepare the extract because the simple method and gives more practical yield as compare to other methods of extraction.

2 MATERIAL AND METHODS OF EXTRACTION OF MEDICINAL PLANTS[8]

2.1Selection and Authentication of plant

The entire plant was selected and authenticated at suitable authentication center.

2.2 Standardization of crude drug[9]

The crude drug was standardized on the basis of following parameters i.e. loss on drying, total ash, ash value, acid insoluble ash, water soluble ash, water soluble extractive, alcohol soluble extractive.

2.3 Preparation of extract by using different solvent

Crushed powder of *Cucurbita maxima* was prepared by drying fresh seeds and commutated by using grinding mill (Unicon – CM/L-7852591). Methanol, n –hexane, Acetone, water solvents were used for extraction.

Methods of Extraction of Medicinal Plants[8]

2.3.1. Maceration

In this process, the whole or coarsely powdered crude drug is placed in a stopper container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing.

The seeds of Cucurbita maxima were extracted by using an maceration extraction.2gm powdered drug of Cucurbita maxima was taken in four different beakers contain 10 ml water, methanol, acetone, n-hexane was added in each beaker. The beakers was sealed with aluminium foil for 4-7 days. After 7 days the extract was filtered and collected in different petri plates. The pertiplates were kept aside for evaporation at room temperature.

2.3.2. Hot Continuous Extraction (Soxhlet)

In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus (Figure 2). The extracting solvent in flask A is heated, and its vapors condense in condenser D. The condensed extract and drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into fl ask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This effects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.

The seeds of Cucurbita maxima were extracted by using an soxhlation extraction.10gm powdered drug Cucurbita maxima four was placed in porous bags or thimble was made up of filter paper was placed in body of soxhlet aparatus contain water, methanol acetone, n-hexane. The condensed extractant drips into the thimble containing crude drug. When the level of liquid in chamber rises to the top of siphon tube, the active constituents of chamber siphon tube into the flask, thus emptying the body of extractor. This alteration of filling and emptying the body of extractior goes on continuously. The soluble active constituents of a drug remain in the flask while solvent is repeatedly volatilized. The process of filling and emptying of the extractor were repeated until the drug was exhaustion.

2.3.3. Ultrasound Extraction (Sonication)

The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like extraction of Rauwolfia root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but

known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules. The seeds of Cucurbita maxima were extracted by using an Ultra-sonication, extraction.10gm powdered drug Cucurbita maxima was taken in four different beakers contain 50 ml water, methanol acetone, n-hexane was added in each beaker. The beakers were kept in sonicator for Sonication. Extraction process carried out for 15 minutes respectively. The extract was filtered and collected in different petri plates. The perti plates were kept aside for evaporation of solvent at room temperature and then collected extract was stored in refrigerator.

2.4 Identification of Phytochemical constituents present in the extract

Phytochemical constituents present in the extract were determined by performing test for alkaloids, steroids, carbohydrate, saponins, flavanoids, cardiac glycosides, Anthraquinone glycosides.

RESULT AND DISCUSSION

3.1 Authentication of plant

The fresh seeds of the plant were collected from the Satara city, Maharashtra and authenticated at of Yashvantrao Chavan Institute of Science, Satara.

3.2 Standardization of crude drug

The crude drug was standardized on the basis of following parameters i.e. loss on drying, total ash, ash Value, acid soluble ash, water insoluble ash, water soluble extractive, alcohol soluble extractive, ether soluble extractive. The results of all the standardization parameters are as given in table 1. All the standardization parameters were found to be within the standard limits indicating purity of crude drug.

Table 1: Standardization parameters for *Cucurbita maxima*.

Parameters	Values %(w\w)
Ash value	2.90%
Acid insoluble ash	0.215%
Water soluble ash	0.677%
Loss on drying	7.232%
Water soluble extractive value	14.9%
Alcohol soluble extractive value	8.7%

Parameters % W/W* 1. Ash values Total ash 3.121 Acid insoluble ash 0.279 Water soluble ash 0.689 2. Loss on drying 7.995 3. Water soluble extractive value 15.8 4. Alcohol soluble extractive value 9.

3.2.1. Preparation of extract of *Cucurbita maxima* by maceration extraction method

The seeds of Cucurbita maxima were extracted by using an maceration**.** Percentage yield of all solvent extract was shown in table no.2.

Table 2: Percentage yield of extract by maceration extraction method.

Sr. No.	solvent	% practical yield (w/w)
1	Water	10.5%
2	Methanol	16.5%
3	Acetone	29.4%
4	n-hexane	16.5%

3.2.2 Preparation of extract of *Cucurbita maxima* by Soxhlet extraction method

The seeds of Cucurbita maxima were extracted by using soxhalation. Percentage yield all solvent extracts were shown in table no.3.

Table 3: Percentage yield of extract by Soxhlet extraction method.

Sr. No.	solvent	% practical yield (w/w)
1	Water	3.6%
2	Methanol	5.2%
3	Acetone	39.4%
4	n-hexane	20.2%

3.2.3. Preparation of extract of *Cucurbita maxima* by Soxhlet extraction method

The seeds of Cucurbita maxima were extracted by using an Ultra-sonicator**.** Percentage yield all solvent extract was shown in table no.4.

Table 4: Percentage yield of extract by Sonication extraction Method.

Sr. No.	Time	solvent	% practical yield (w/w)
1	15 min	Water	5.8%
2	15 min	Methanol	8%
3	15 min	Acetone	11.4%
4	15 min	n-hexane	5%

Comparative study of extraction techniques used for C. maxima

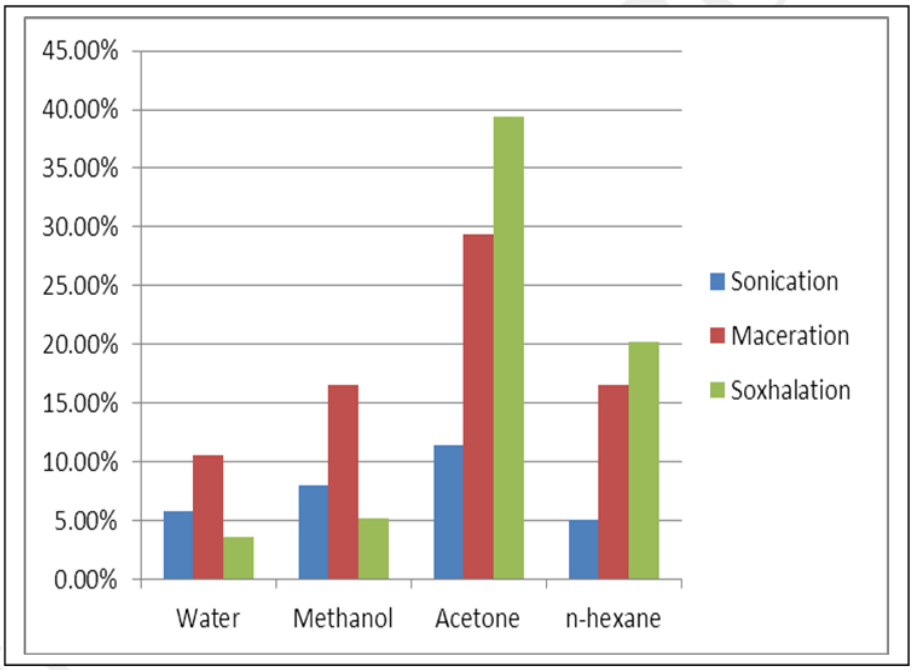
Table no 5: Percentage of extract by using different extraction techniques and different solven.

	Sonication	Maceration	Soxhalation
Water	5.8%	10.5%	3.6%
Methanol	8%	16.5%	5.2%
Acetone	11.4%	29.4%	39.4%
n-hexane	5%	16.5%	20.2%

From the above observation table

The Soxhlet extraction process was given highest percentage of extract as compared to the sonication and maceration method.

B) By Statistical Analysis.



From the above statistical analysis

The sonication and maceration process were given less percentage of extract as compared to Soxhlet extraction technique method.

3.4 Phytochemical constituent present in the extract

Phytochemical constituent present in the extract exhibited the entire test for alkaloids, steroids, carbohydrate, saponins, flavanoids, cardiac glycosides, Anthraquinone glycosides, positive sign indicating that the constituents were present in the extract (Table 4).

Table 6: Test for different Phytochemical constituents.

	Methanol	Acetone	N-Hexane	Water
Alkaloids				
Dragendroff's test	+	+	-	+
Wagners test	+	+	-	+
Mayers test	+	+	-	+
Hagers test	+	+	-	+
Steroids				
Libermann-buchard test	-	-	-	-
Salkowaski test	-	-	-	-
Carbohydrates				
Molish test	+	+	-	+
Fehling test	+	+	-	+
Benedicts test	+	+	-	+
Bradford's test	+	+	-	+
Saponins				
Foam test	+	+	-	+
Flavanoids				
Shinoda Test	+	+	-	+
Cardiac Glycosides				
Legal's test	-	-	-	-
Keller- killani test	-	-	-	-
Anthraquinone glycosides				
Brontrager's test	+	+	-	-

CONCLUSION

By comparative extraction techniques study we concluded that the Soxhlet extraction technique gives maximum percentage yield of extract and phytochemical constituents were found maximum in the Alcoholic and acetone extracts.

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