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## Standardization of Ethanolic Extract of *Cucurbita Maxima* Seed

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### ABSTRACT

Standardization is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance program for production and manufacturing of herbal drugs preparation of any herbal formulation identification, evaluation and standardization is rudimentary identification involves the morphology, microscopy parameter of plants, evaluation and standardization of herbal drugs includes physical, chemical and biological parameters. These parameters are crucial for preparation of accurate and potent formulation. The present communication attempts to investigate pharmacognostical and phytochemical details of *Cucurbita maxima*, (Cucurbitaceae). The Preliminary phytochemical analysis revealed presence of carbohydrates, alkaloids, flavonoids, saponins, proteins and amino acids in alcoholic extract. HPTLC studies reveal that alcoholic extract gives 3 spots and alcoholic extract depicts 5 spots on the TLC plate in Butanol: acetic: water solvent system with Ninhydrin as spraying agent and 3 spots with vanillin as spraying agent and with butanol: phenol: water (6:1:1) 4 spots were seen with Ninhydrin as spraying agent and 2 spots were seen with vanillin as spraying agents. The GC/MS of pet ether methyl ester showed number of peaks. Out of which 3 highest peaks in descending order were taken into consideration. OSAZONE were formed which showed needle shaped crystals of glucaosazone. The study revealed specific identities for which may play a key role in identification of plant and can be useful in standardization of the herbal drugs.

**Keywords:** *Cucurbita maxima*, ethanolic extract, *Cucurbita maxima* seed, Cucurbitacins.

### INTRODUCTION

“Health for all” is a dream and a goal which humanity at large shares and strives for. Unfortunately, it has now been proven without doubt that modern pharmaceuticals are and will remain out of reach for a large proportion of the human population for the foreseeable future. This has created an appreciation and a need for the use of other sources of human knowledge to provide common health benefits. Alternative and traditional medicines, largely herbal in nature, are now regarded as important but underutilized tools against disease which have better compatibility and lesser side effects (Mohmmad *et al.*, 2008; Kamboj, 2000). Considering the adverse effects of synthetic drugs, the Western population is looking for natural remedies which are safe and effective (Dubey *et al.*, 2004).

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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 (Modak *et al.*, 2007; Patra *et al.*, 2010). The genus *Cucurbita*, indigenous to the western hemisphere, is comprised of five domesticated species (Loy, 2004). Of the 50 common varieties of cucurbita throughout the world, there are 2 general categories: the pumpkin and the squash. *Cucurbita pepo* L., *Cucurbita maxima* Duchesne, and *Cucurbita moschata* Duchesne represent economically important species cultivated worldwide (Loy, 2004; Milani *et al.*, 2007).

The cucurbitacins are a group of bitter tasting, highly oxygenated, mainly tetracyclic, triterpenic plant substances derived from the cucurbitane skeleton [19-(10→9β)-abeo-10α-lanost-5-en]. The most important pharmacological activities of cucurbitacin are purgative activity and their cytotoxic and antitumoral, hepatoprotective, anti-inflammatory, antifertility in female mice and stomachic among other properties. They can also play other biological roles such as plant growth regulators and insect feedant or antifeedant (Miro, 1995).

A system to ensure that every packet of medicine that is being sold has the correct substances in the correct amount and will induce its therapeutic effect this is known as standardization (Ekka *et al.*, 2008). A medicinal herb can be viewed as a synthetic laboratory as it produces and contains a number of chemical compounds. These compounds, responsible for medicinal activity of the herb, are secondary metabolites. Complete phytochemical investigations of most of the medicinally important herbs of India have not been carried out so far. This would be beneficial in standardization and dose determination of herbal drugs. Further, there should be a quality control test for the entire preparation to ensure the quality of the drug (Kamboj, 2000). Each traditional system of medicines has their own method of standardization for assuring quality most in human linguistic terms. This method of evaluation has to be taken into consideration in standardization of herbal medicine (Patra *et al.*, 2010).

## MATERIAL & METHOD

### Collection, Authentic and Processing of Plant Material

The seeds of *Cucurbita maxima* were collected from local market of Bhopal in month of September-October which was authenticated by Dr. H. B. Singh Scientist and head. Raw material herbarium and museum NISCAIR, New Delhi (Herbarium no. BUPH-1636/234). The seeds were washed and dried in shade. The material was added in mechanical grinder for size reduction and store in air tight container.

### Extraction and Fractionation Procedure

The powdered crude drug (seed) was extracted with petroleum (pet) ether by cold extraction method (using Soxhlet apparatus). The Soxhlet process is useful for the exhaustive extraction of plant material with a particular solvent, e.g. for defatting or where 100% of a particular component is desired. It is useful where exhaustive sequential extraction with a series of

solvents of increasing polarity is desired, e.g. hexane, chloroform, methanol, water (Mukerjee, 2002).

The crude drug was extracted using pet ether, ethanol. Extraction using pet ether was done to defat the drug and later on ethanol was used as solvent for extraction. In Soxhlet apparatus, in which the vapour passes through the side tube and reflux returns to the extraction chamber where the solution collects. As this takes place, the liquid level will also rise in the return tube; when the liquid reaches the liquid reaches the top of the return tube, a siphon is set up and contents of the extraction chamber are transferred to the flask. This method is referred to as continuous cold extraction, since vapour is by passed through the side arm and does not enter the extraction chamber directly (Carter, 1986).

### Physicochemical Parameter of Crude Drug

The different physicochemical parameter was used for determining the purity of crude drugs as the determination of moisture content, determination of ash values (determination of total ash, determination of acid insoluble ash, determination of water –soluble ash), determination of solvent extractive values (determination of alcohol –soluble extractive, determination of water soluble extractive, determination of ether –soluble extractive {fixed oil content}).

### Phytochemical Studies

The extract obtained was then subjected to various qualitative tests for the identification of various plant constituents like alkaloids, carbohydrates, proteins and amino acids, flavonoids, saponin, fixed oil and steroids.

### Qualitative and Quantitative Methods of Traditional Medicines Chromatographic Methods

#### Thin Layer Chromatography (TLC)

TLC is a simple, low-cost, versatile and specific method for the identification of herbal medicines (Patra *et al.*, 2010). The unique feature of picture-like image of TLC supplies an intuitive visible profiling.

Thin layer is a mode of liquid chromatography in which the sample is applied as a small spot or streak to the origin of a thin sorbent layer supported on a glass, plastic or metal plate .the mobile phase moves through the stationary phase by capillary action ,sometime assisted by gravity or pressure (Chatwal, 2002).

#### HPTLC

Nowadays HPTLC is a routine analytical technique. It has also been reported that mobile phases of pH 8 and above can be used for HPTLC. Another advantage HPTLC is the repeated detection (scanning) of the chromatogram with the same or different conditions. Consequently, HPTLC has been investigated for simultaneous assay of several components in a multi-component formulation. With this technique, authentication of various species of plant possible, as well as the evaluation of stability and consistency of their preparations from different manufactures (Patra *et al.*, 2010).

### Methyl Esters

Lipids play a major role in biological functions due to their presence in all cells. Fatty acids are commonly found in natural product extracts and have been shown to interfere with noncellular assays (Barry, 1955). Fatty acid esters are the molecules containing non polar groups and may be used in drug development for lipid based formulations. Identification of methyl esters can be done by GC/MS.

### Osazone

Osazone are a class of carbohydrate derivatives found in organic chemistry formed when sugars are reacted with phenyl hydrazine (Furniss, 2006). The reaction involves formation of a pair of phenylhydrazone functionalities, concomitant with the oxidation of the hydroxymethylene group adjacent to the formyl center. The reaction can be used to identify monosaccharide.

## RESULTS AND DISCUSSION

The physicochemical parameters like moisture content, total ash value, acids insoluble ash, extractive value was determined and results obtained indicates that the seeds collected were not adulterated (Table 1 and 2).

**Table 1:** Ash Value.

Total Ash	Acid insoluble Ash	Water soluble Ash
7.5%	3.0%	3.6%

**Table 2:** Extractive Value.

Water Soluble Extractive Value	Alcohol Soluble Extractive Value	Pet Ether Soluble Extractive Value
4 g w/w	3.5g w/w	37.6 g w/w

Phytochemical testing for the presence of various chemical constituents was performed using standard tests and procedures. The data reveals the presence of tannins, carbohydrates, glycosides, alkaloids, volatile oils, saponins, proteins and flavonoids (Table 3).

**Table 3:** Phytochemical Studies.

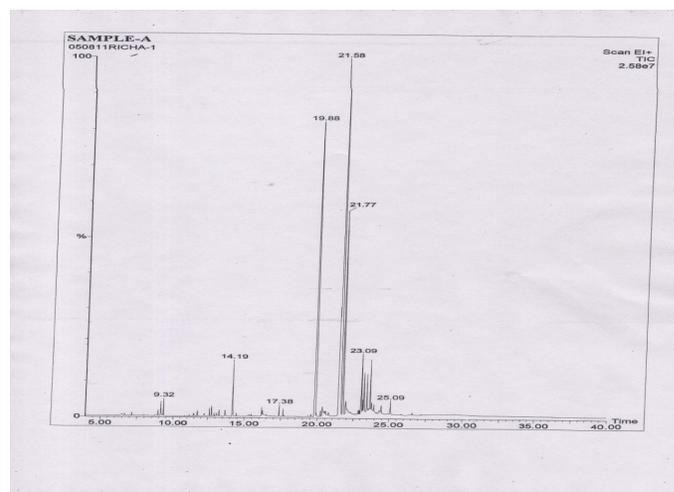
Chemical Class	Chemical Test	Ethanollic Extract
Carbohydrates and Glycosides	Dragendorff's reagent	-ve
	Molisch's reagent	+ve
	Fehling's test	+ve
	Barfoed's reagent	-ve
	Benedict's reagent	+ve
Phytosterols	Legal's test	+ve
	Salkowski's test	-ve
Fixed oil and fats	Spot test	+ve
	Foam test	-ve
Saponins	Ferric chloride solution	+ve
	Ninhydrin reagent	+ve
Phenolic compounds and Tannins	Molisch's reagent	+ve
Gums and mucilages		

Table 4 shows the TLC report of the extract, in which test solution was Ethanolic solution of the *Cucurbita maxima* seed and solvent system was: Butanol: acetic acid: water (4:1:1); Methanol: water (9:1); Benzene: Chloroform (1:1), Chloroform (100%); Methanol (100%); Butanol: acetic acid: water (6:1:1). Results were visualized on only Butanol: acetic acid: water (4:1:1). The GC-MS

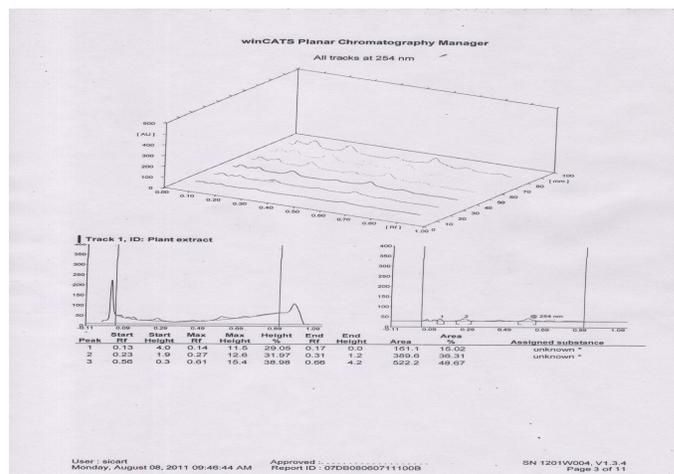
analysis of *Cucurbita maxima* seed extract showed the presence of Individual unsaturated fatty acids which were identified from  $R_f$  peak area and by comparison of the data those reported in the literature. The GC/MS of pet ether methyl ester showed number of peaks. Out of which 3 highest peaks in descending order were taken into consideration. GC/MS analyses of the fatty acid methyl esters also showed the degree of unsaturation. Linoleic acid was found to be the dominant fatty acid 46.12% followed by oleic acid 28.79%. Palmitic acid 12.31% was the major saturated fatty acid. The HPTLC analysis of ethanolic extract of *Cucurbita maxima* seed extract showed 3 peaks of cucurbits with percent area around 15.02, 36.31 and 48.67 (Fig 2). The Osazone formation of *Cucurbita maxima* seed showed needle shaped crystals of glucaosazone (Fig 3).

**Table 4:** TLC of Ethanolic Extract of *Cucurbita Maxima* Seeds.

Name of Extract	Solvent system	Spray reagent	No. of spots		$R_f$ value	
			Ninhydrin	Vanillin	Ninhydrin	Vanillin
Ethanolic extract of <i>Cucurbita Maxima</i> Seed	Butanol: Acetic acid: Water (4:1:1)	Ninhydrin /Vanillin -H <sub>2</sub> SO <sub>4</sub>	5	3	0.19 0.28 0.38 0.5 0.63	0.18 0.30 0.76



**Fig. 1:** GC/MS Reports of Methyl Esters.



**Fig. 2:** HPTLC Profile.



Fig. 3: Crystals of Glucosazone.

### CONCLUSION

The qualitative analysis of drug shows presence of steroids, carbohydrates, unsaturated fatty acids and saturated fatty acids in the extract. The  $R_f$  value of TLC and peaks of HPTLC showed the presence of various active chemical constituents. The methyl esters were formed and fatty acids were determined using GC-MS techniques.

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