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A Review of Physio Chemical & Bioactive Profiling by Various Chromatographic Techniques in Cucurbita Maxima

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ABSTRACT:
Cucurbita maxima have been a traditional plant in India. Irrspective of its nutritional value it has proven pharmacological value. The current study focus mainly on collection of articles related to various chromatographic isolation studies on cucurbita maxima. This study gaves a wide exposure of various parts of cucurbita used with respective to various parts of cucurbita used with respective to various chromatographic techniques leading to the profile of biologically active compounds.

Keywords: Cucurbita maxima, Chromatography, Isolation compounds, Anthelmintic, Anti-bacterial.

AIM:
The aim of the study is to review and profile the various physiochemical and bioactive properties using chromatographic techniques in cucurbita maxima(Pumpkin).

OBJECTIVE:
To review the various parts of pumpkin using different kinds of extract exerting individual pharmacological activities.
To review and categorize the isolation of bioactive compounds by chromatographic techiques and its identification.
To profile its physiochemical properties.

INTRODUCTION:
Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. India is a vast repository of medicinal plants that are used in traditional medical treatments. WHO too has not systematically evaluated traditional medicines despite the fact that it is used for primary health care by about 80% of the world population. However, in 1991 WHO developed guidelines for the assessment of herbal medicine. Suggestions for herbal medicine standardization are outlined. Safety of some herbal ingredients has been recently called into question, in part because of the identification of adverse events associate d with their use and, increasingly, because of the demonstration

of clinically relevant interactions between herbs and prescription drugs. But in the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects [1].

The pumpkin (Cucurbita spp.), one of the most popular vegetables consumed in the world, has been recently recognized as a functional food. Pumpkin seeds, generally considered agro-industrial waste, are an extraordinarily rich source of bioactive compounds with interesting nutraceutical properties. In recent years, several studies have highlighted the health properties of pumpkin seed oil against many diseas es, including hypertension, diabetes, and cancer [2].

These species possess a higher number of proteins, phytosterols, unsaturated fatty acids, vitamins (like carotenoids, tocopherolsand microelements (e.g., zinc). Fruits, seeds and leaves from various Cucurbita members (pumpkin, watermelon, melon, cucumber squash, gourds, etc.) possess different pharmacological aactivities

1. BOTANICAL ASPECTS

Botanical name: Cucurbita maxima Duchesne.
Synonyms: Cucurbita pepo var. maxima (Duchesne) Delile.
Plant Family: Cucurbitaceae. Plant Form: Climbers.
Leaves: Ovate, oblong, 5-7 lobed, dentate, cordate, hairy and coarse.
Flowers: Male flowers axillary and solitary, yellow, corolla gamopetalous, campanulate
Fruit: A pepo, very large globose pale yellow-orange. Time: August-September
Significance: Cultivated everywhere for its fruits which are used as vegetables.

PHARMACOLOGICAL ACTIVITIES.

Anticancer

Anticancer Activity- Anticancer activity of methanol extract of Cucurbita maxima against Ehrlich as-cites carcinoma. Cancer is a pathological state involving uncontrolled proliferation of tumour cells. The study was carried out to investigate the antitumor potential of MECM (methanol extract of Cucurbita maximus) against EAC (Ehrlich Ascites Carcinoma) bearing mice. EAC is a very rapidly growing carcinoma with very aggressive behaviour.

Analgesic activity

The acetic acid induced within method is an analgesic behavioural observation assessment method that demonstrates a noxious stimulation mouse. The test consists of injecting the 0.7% acetic acid solution intraperitoneally and then observing the animal for specific contraction of body referred as ‘writhing’. [Moumita Panda,Thin layer chromatograpic studies and in vitro free radical scavenging effects of Cucurbita maxima leaf extracts;

Antioxidant activity

Spotted on pre-coated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar of the extract. The plates were dried at room temperature and were sprayed with 0.02% 1, 1-diphenyl- 2-picryl hydrazyl (DPPH) in ethanol.

Immunogenic Activity

Antigenotoxic spinasterol from Cucurbita maxima flowers. The antigenotoxic constituent of squash flowers was isolated by solvent partitioning and repeated vacuum liquid chromatography.

The flower of Cucurbita maximus contains several sterols which are responsible for the antinogenic activity.

Diuretic Activity

Diuretic activity of seeds of Cucurbita maxima duchesne in albino wistar rats. The seeds of Cucurbita maxima Duchesne are used traditionally as diuretics and other urinary diseases.

The concentration of Na+ and K+ in urine was determined by flame photometer. The volume of urine and Na+ and K+ concentration of test group was compared with the control group.

The results revealed that the aqueous extract of seeds of Cucurbita maxima showed significant increase in urine volume when compared to control group. But the excretion of Na+ and K+ in urine was not significantly increased in drug treated group when compared to control group.

Antidiabetic Activity

Pumpkin is most widely studied with regard to its antidiabetic effect and the fruit pulp and seeds of this plant have shown hypoglycemic activity in normal animals and alloxan-induced diabetic rats and rabbits. Both common and sugar-removed pumpkin powder showed a significant reduction in blood glucose and an increase in plasma insulin and protected the diabetic nephropathy

Hypoglycemic activity

Water-extracted pumpkin polysaccharides was demonstrated and excelled Glibenclamide in alloxan-induced diabetic rats.

Antihyperglycemic activity

Antihyperglycemic activity of water-extracted pumpkin polysaccharides was observed in normal rats. Crude polysaccharide from pumpkin fruit was reported to reduce branched chain amino acid and have better effect on normal rats than on alloxan-induced diabetic rats.

We report that protein-bound polysaccharide can obviously increase the levels of serum insulin, reduce the blood glucose levels and improve tolerance of glucose.

The hypoglycemic effect of big dose protein -bound polysaccharide group (1000 mg/kg body weight) excelled that of small dose protein-bound polysaccharide group (500 mg/kg body weight) and Glibenclamide group.

Eighteen amino acids were identified to be components of the protein bound polysaccharide but the relationship between the contents of amino acids and hypoglycemic activity of pumpkin protein-bound polysaccharide is not clear.

Antibacterial activity.

There were reports on broad-spectrum antimicrobial activity of pumpkin extracts. Pumpkin oil inhibits Acinetobacter baumanii, Aeromonas veronii, Biogroup sobria, Candida albicans, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterica subsp. enterica serotype typhimurium, Serratia marcescens and Staphylococcus aureus at the concentration of 2.0% (v/v).

Anthelmintic

Pumpkin seed was found to be a vermifuge and was eaten fresh or roasted for the relief of abdominal cramps and distension due to intestinal worms.

The effect of water extracts of pumpkin seeds in the treatment of puppies experimentally infected with heterophyiasis could obtain promising results and combined extracts of areca nut and pumpkin seeds gave an excellent result than when given either extract alone.

An Anthelminthic effect was reported at the minimum inhibitory concentration of 23 g of pumpkin seed in 100 ml of distilled water in preclinical studies.

REVIEW OF LITERATURE

Daisy.P etal stated that Cucurbita maxima and its native is North America. They naturally have a thick, orange or yellow shell. Pumpkins are broadly grown for commercial use, and are used both in food and recreation. In India, it is most consuming vegetables. Pumpkins are considered to be a fruit and it contains 90 percent water. Pumpkins have antioxidant beta-carotene, which help to improve the immune function and can reduce the risk of diseases like heart disease and cancer. (9,10). Cucurbita maxima commonly known as Pumpkin has been used for various ailments cold, benign prostatic hypertrophy etc. This species are believed to have antitumor effect but its mechanism for activity remains to be elucidated.

In this study compound present in the methonal extracts of pumpkin was identified through HPLC and Gc/Ms analysis and its mechanism of action was identified through docking analysis.

Materials and methods

Collection of Plant Material Cucurbita maxima seeds were collected from Tiruchirappalli District Tamil Nadu, India. The seeds were shade-dried and coarsely powdered. Preparation of Plant Extracts 500gms of seed powder was taken in an aspirator bottle; 1.5 litre of Methanol was used and the mixture was shaken occasionally for 72 hours. Then the extract was filtered. This procedure was repeated three times and all extracts were decanted and pooled. The extract was filtered before drying using Whatmann filter paper no.2 on a Buchner funnel and the solvent was removed by vacuum distillation in a rotary evaporator at 40.C.. Mobile Phase of 65 Volumes of methanol and 35 Volumes of water were used. GC-MS analysis The GC- MS analysis was performed on a combined GC-MS instrument using a HP-5 fused silica gel capillary column. The method to perform the analysis was designed for both GC and MS using the X Caliber Software provided with the machine. A 1 µl-aliquot of sample was injected into the column using a PTV injector whose temperature was set at 275 degree Celsius. The GC program was initiated . The chromatogram and spectrum of the peaks were visualized using Qual Browser software. The particular compounds present in the samples were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998) library.

RESULTS:

Desmosterol was identified as a predominant compound present in the methanol seed extract of Cucurbita maxima.

Celebs.J, etal stated that This Study was done to investigate the phytochemical and antimicrobial activities of pumpkin flowers extracts (ethanolic, chloroform and ether extract –by using GC-MS methods to degrade the active components in the ethanol extracted flowers. Nine constituents were degraded by Gas

Chromatography Mass Spectrophotometry (GC-MS) analysis Materials and Methodology The Plant A pumpkin Flowers was collected from the local farm of Khartoum city in February 2012; authenticated, dried at shade and reduced to fine powder using pestle and mortar in the laboratory as described by Bean AR [6]. The powder was stored dry, and used as the stock sample for further analyses. Instruments and Chemicals A wide range of instruments and chemical were used The phytochemical compounds in the flowers of pumpkin extracted with ethanol screened by GC-MS method, 9 bioactive phytochemical compounds were identified. These different active phytochemicals have been found to possess a wide range of activities, which may help in the protection against numerous diseases. Activity of the Identified Phytochemicals in Pumpkin Flowers Palmitic acid:

Conclusion As a consequence of all these bioactive nine compounds and their important biological activities, Allah Almighty had chosen the pumpkin tree for the prophet Yunus (pbuh) because of its benefits and usefulness in the large scale in recovering health and strength, and because of the presence of several beneficial active ingredients. However, isolation of individual phytochemicals and subjecting it to biological activity will definitely give fruitful results Alhassan, etal stated that The roots of Cucurbita pepo were investigated with the aim of identifying bioactive constituents with therapeutic potentials. To achieve this, the roots of Cucurbita pepo were harvested, washed, air dried and grounded into the powder. The ground materia was extracted using hexane, ethyl acetate and methanol. The extracts were fractionated using column chromatography and fractions were monitored using thin layer chromatography. The fractions were characterised using proton nuclear magnetic resonance and carbon-13 nuclear magnetic resonance spectroscopy. Phytochemical screening showed that the extracts contained sterols and terpenoids, alkaloids, resins, flavonoids, saponins and carbohydrates. Nuclear magnetic resonance analysis led to the identification of hexadecanoic acid or palmitic acid, alphaspinasterol and squalene. Some of these compounds may represent a new pharmacological approach in the development of novel and adjuvant therapy for several medical conditions.

METHOD:

The roots of Cucurbita pepo were collected . The roots were washed and air dried. Once completely dry, the roots were ground to powder using a blender. The ground roots were stored in closed containers at room temperature until required. Extraction and phytochemical screening: The powdered material (350 g) was introduced into a Winchester bottle and macerated successively for 48 h with 700 ml of hexane, ethyl acetate and methanol. The extracts were filtered into clean glass jars using Whatman No.1 filter papers and allowed to dry in fume hood. All extracts were subjected to phytochemical analysis.

RESULTS AND DISCUSSION

Phytochemical screening of extracts showed that the root extracts of Cucurbita pepo contained sterols and terpenoids, alkaloids, resins, flavonoids, saponins and carbohydrates.

PA.Ekeocha etal ,stated that C. pepo leaves extracted with 90% methanol by maceration with continuous shaking at room temperature for three days. Thin-layer chromatography (TLC), (analytical and preparative) high- performance liquid chromatography, and liquid mass chromatography used for isolation and identification. Preparative HPLC was carried out for isolation of present peaks of hexane fraction. Preparative HPLC conditions

RESULTS AND DISCUSSION:

Fractionation of Extracts For full phytochemical profile screening for a given plant, fractionation of the crude extract had been recommended so that the main class of the plant constituents will be isolated from each other according to the differences in the polarity and solubility before chromatographic analysis is performed since crude extract contains diverse classes of chemical constituents with various polarities.20 Fractionation of crude extract is done with a series of solvents of increasing polarities (hexane, chloroform, ethyl acetate, and n-butanol). Preliminary phytochemical examination of leaves extracts fractions revealed the presence of triterpenes in hexane fraction that is characterized by coloring with brownish- black color

CONCLUSION:

The results of the current study isolate and identify two cucurbitacins (cucurbitacin B and cucurbitacin E), in addition to the detection of beta-sitosterol in methanolic extract of C. pepo leaves, where LC mass/mass result is reasonable, and HPLC analysis showed matching between samples and standards HPLC of standards: Cucurbitacin B, Cucurbitacin E, beta-sitosterol, and Stigma sterol.

Haider M.Kadhim etal, stated that The present study assessed the different solvent extracts of C. maxima leaf for thin layer chromatography (TLC) and also evaluated their in vitro free radical scavenging potential by 1, 1- diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay. The petroleum ether extract yielded maximum spots in TLC. All of the extracts exhibited potent in vitro free radical scavenging activity that increased with extract concentration. The methanol extract was found to be the most potent in this regard, followed by the chloroform and petroleum ether extracts

Materials and methods

Plant material: The mature leaves of Cucurbita maxima powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

Preparation of plant extracts: The dried powdered material was defatted with petroleum ether (60-80°C). The defatted powdered material thus obtained was further extracted with chloroform and methanol for 72 h. The solvent was distilled off in reduced pressure and resulting semisolid mass was vacuum dried to yield the dry extracts and the percentage extractive values were accordingly

2.42 % w/w and 6.54 % w/w respectively. The preliminary phytochemical analysis was performed for all three extracts to identify the phytoconstituents present .

Thin layer chromatographic studies: Each solvent extract was subjected to thin layer chromatography (TLC) as per standard one dimensional ascending method.

Results and discussion

Preliminary phytochemical studies showed the presence of steroids in the petroleum ether extract, triterpenoids in chloroform extracts whereas triterpenoids, tannins, glycosides and carbohydrates in the methanol extract form C. maxima leaf. The present thin layer chromatographic studies revealed the presence of maximum constituents in the chloroform extract, as it exhibited maximum numbers of well resolved spots.

Moumita Panda etal, stated that Cucurbita pepo (pumpkin), a Cucurbitaceae membered plant, is considered one of the oldest cultivated plants. Thin-layer chromatography (TLC), (analytical and preparative) high- performance liquid chromatography, and liquid mass chromatography used for isolation

and identification of two Cucurbitacins from C. pepo (pumpkin) leaves methanolic extract and detection of phytosterols. Cucurbitacins are triterpenes based structure isolated from many members of Cucurbitaceae families and other plants. Cucurbitacins exhibit polar properties, so they are isolated from plant extracts, which are extracted with methanol. Cucurbitacins exhibit anti-cancer activity, anti-atherosclerotic activity, and anti-arthritis activity, so Cucurbitacins may be an important lead molecule for future new medicinal preparation.

METHOD:

Among the various methods for separating plant constituents, the chromatographic procedure is the one of the most commonly used techniques of general application (15). Thin layer chromatography (TLC) involves the separation of mixtures of organic compounds on thin layers of adsorbents that are usually coated on glass, plastic, or aluminum sheets; and this particular technique is the easiest, cheapest and most widely used method for the characterization of natural products and their preparations. C. pepo leaves extracted with 90% methanol by maceration with continuous shaking at room temperature for three days. Thin-layer chromatography (TLC), (analytical and preparative) high-performance liquid chromatography, and liquid mass chromatography used for isolation and identi cation of two Cucurbitacins from C. pepo (pumpkin) leaves methanolic extract and detection of phytosterols.β-Sitosterol Isolated and Identi ed from Cucurbita pepo Leaves Preparative HPLC was carried out for isolation of present peaks of hexane fraction. Identification by LC/Mass/Mass (Liquid/mass/mass chromatography)

RESULTS AND DISCUSSION

The TLC analysis revealed the presence of phytosterol and terpenes.

CONCLUSION

The results of the current study isolate and identify two cucurbitacins (cucurbitacin B and cucurbitacin E), in addition to the detection of beta-sitosterol in methanolic extract of C. pepo leaves, where LC mass/mass result is reasonable, and HPLC analysis showed matching between samples and standards. Emir Tosin etal stated that The objective of this study was to develop a rapid, economic, and efficient method for simultaneous selective isolation, separation, and purification of cucurbitacin D and I . A Rich fruit juice via reversed-phase flash chromatography combined with HPLC. The chloroform extract of the fruit juice was fractionated with flash chromatography Cucurbitacin D and I were collected automatically by the fraction collector. The fractions containing the same compounds were pooled and lyophilized. The purified cucurbitacin D and I compounds were identified by NMR, LC-MS, and UV spectra analysis. The results suggest that the applied procedure is simple, quick, and highly efficient. The HPLC method was found to be linear, accurate, precise and rugged for the quantification of the cucurbitacins studied.

Different techniques such as Flash Chromatography (FC) and HPLC may be used for the isolation and purification of plant extracts. FC is a rapid and economical method for the separation of mixtures at relatively high flow rates. FC offers good separation and can be used in both normal phase and reverse phase separations, but to the best of our knowledge, the use of FC has barely been studied to separate cucurbitacin species [23, 24].

MATERIALS AND METHODS

Materials, instruments, standards and reagents Extraction and isolation of cucurbitacins The ripe fruits

were pressed. The juice was collected and strained. The juice was filtered through a double layer cheesecloth, then through filter paper, and dried in the vacuum oven at 23 [degrees]C. To prevent degradation of secondary metabolites, the residue was stored at about-18 [degrees]C prior to utilization for extraction.

Five grams of dried residue was dissolved in 50 ml deionized water. This aqueous solution was extracted three times with 50 ml hexane at 40 [degrees]C for 6 h to remove waxes, pigments, high boiling terpenes, apolar fatty acids, and lipids. After extraction, the phases were separated and stored at-18 [degrees]C until analysis.

The remaining aqueous phase (49 ml) was extracted with chloroform (50 ml), which has high affinity for cucurbitacins. The extraction with chloroform was performed three times at room temperature for 6 h. The extract was then filtered and concentrated to 10 ml by the rotary evaporator under reduced pressure at 40 [degrees]C. This organic phase contained a mixture of partially purified cucurbitacins.

FC was used for the fractionation of the chloroform extract to obtain pure cucurbitacins in target fractions. The solvent system used for FC was chosen from TLC separation experiments. For this purpose, the optimum solvent system ratio giving the best separation of Cu D and Cu I was determined from several solvent systems. Standards and test samples were spotted on TLC silica gel

60 [F.sub.254] aluminum sheets (Merck, Darmstadt, Germany). The solvent system which gave the best separation was a chloroform-acetone-methanol (77:10:13; v/v/v) solution. The Rf values for Cu D and Cu I were found to be 0.61 and 0.69, respectively.

HPLC analysis was carried out at constant column temperature (40 [degrees]C), and a 10 [micro]l injection volume was taken for qualitative/quantitative analysis.

The isolated compounds were further identified by spectroscopic methods including UV, LC-MS and NMR. Isolated Cu D and Cu I samples were dissolved in ethanol and determined by HPLC-DAD according to the method described above on the basis of retention time and by comparison of UV spectra of the standards.

RESULTS AND DISCUSSION

The isolated cucurbitacins exhibit a wide array of in vitro and in vivo pharmacological effects, including anti-tumor activity.

Sandeep Singh Rana etal stated that , The edible pumpkin fowers is analysed for the physicochemical, biochemical properties, proximate analysis, antioxidant activities, anthocyanin content and fatty acid profling.

Among several fatty acids' oleic acid (21%), myristic acid (15.99%) and stearic acid (15.19%) was maximum. The presence of several phytonutrients and fatty acids makes pumpkin flower a potential source of functional food in near future

METHOD:

Fatty acid profling has been done by gas chromatographic method. The retention times of the fatty acids were compared with the standard chromatogram and fatty acids were identifed for the flower sample.

RESULTS:

Eleven different fatty acids were identified in the pumpkin flower (Fig. 3). The concentration percentage of different fatty acids were given in Table 5. The saturated fatty acids (SFA) were mainly Capric acid,

Lauric acid, Myristic acid, Palmitic acid, Stearic acid, Arachidic acid, Heneicosanoic acid, and Behenic acid. Two different monounsaturated fatty acid were (MUFA) Palmitoleic acid and Oleic acid. Polyunsaturated fatty acid as Linoleic acid was the only fatty acid identified in the pumpkin extract. O Heneicosanoic acid (12%) were present in the moderate level. In case of aloe-vera flower, presence of myristic acid and palmitic acid were noticeable [29]. In case of pumpkin seed oil different four fatty acid as palmitic, stearic, oleic, and linoleic was observed .

Srividhya V etal stated that, Cucurbita pepo is one of the good supplement of protein, carbohydrate, minerals and fat. This coupled with high mineral content which is advantageous for Materials and Methods Collection and authentication of plant: The fruit of cucurbita pepo were collected from the surrounding areas of Rasipuram, Tamilnadu, India .The fruit were cleaned, dried in shade and crushed to a coarse powder, stored in an air tight plastic container, until further use. Extraction of fruit material: Coarsely powdered fruit of cucurbita pepo were defatted by using petroleum ether(60–80°C) and then extracted with hydoalcohol using Soxhlet apparatus for about 72 h at 40°C.After that the sediment was filtered with Whatman no.1 filter paper (Whatman Ltd, England). The fruit extract was further concentrated under vacuum using rotary vacuum evaporator (Buchan R-V120, Switzerland) at 40°C. The obtained crude extract was weighed and stored at 4 ◦ C for the further analysis [5]. Preliminary phytochemical analysis The hydroalcoholic fruit extract of Cucurbita pepo was subjected to phytochemical evaluation and identified the various plant constituents present in the test sample both qualitatively and quantitatively. The following studies were carried out in phytochemical analysis. Preparation of sample A small quantity of the extract was dissolved in 5ml of distilled water and then filtered. The filtrate was tested to detect the presence of different phytochemical constituents in the sample. Detection of carbohydrate by Molisch's reagent. Detection of phytosterol and steroids by Salkowski test. Detection of tannins by Lead acetate test Detection of saponins by Foam test.

Detection of triterpenoids by Libermann-Burchard test Procedure :

The total flavonoid content of the HFECP(Hydroalcoholic fruit extract of Cucurbita pepo) was determined by using Aluminium chloride colorimetric method..

Results: The total flavonoid content was determined from the standard Quercetin calibration curve and it was expressed as milligrams of Quercetin equivalents (QE) per gram of extract.

Kamboj, V.P etal stated that Proximate, minerals and anti-nutritional concentration of Pumpkin pulp (Cucurbita pepo) were investigated using standard analytical methods as stipulated by AOAC . The proximate composition (%) showed that pumpkin pulp contained and Carbohydrate by difference 66.647 ± 0.01% .The mineral element were Mg, Ca, Mn, Fe, Cu, Pb, Ni and P & also Na and K with values of 159.01 ± 0.2 and K 160.31 ± 0.1 mg/l00kg were estimated using Flame Emission spectrophotometer.

METHODOLOGY Sample collection and treatment Pumpkins, The seeds were removed from the pulp using knife, then the pulp were washed thoroughly with distilled water and then dried in an oven at 600C for 24 hours and were powdered with amechanical grinder, packaged and kept for further analysis. III. Methodology The sample was subjected to proximate Sodium and potassium were Proximate, Mineral And Anti-Nutrient

RESULTS:

Evaluation Of Pumpkin Pulp (Cucurbita Pepo) determined using Flame Photometry. Phosphorus was determined by the VanodoMolybdate method. Tannins was determined , Phytic phosphorus was determined and oxalate was determined.

RESULTS & DISCUSSIONS:

PARTS OF THE PLANT	CHROMATOGRAPIC TECHNIQUES	ISOLATED COMPOUNDS
1.Cucurbita maxima seeds	Gas Chromatography Mass Spectrophotometry (GC-MS) analysis.	Desmosterol
2.pumpkin Flowers	GC and MS analysis	.Palmitic acid
3.roots of Cucurbita pepo	Thin-layer chromatography.	sterols and terpenoids, alkaloids, resins, flavonoids, saponins and carbohydrate
4. Cucurbita pepo leaves	Thin-layer chromatography (TLC), (analytical and preparative) high-performance liquid chromatography, and liquid mass chromatography.	Beta-sitosterol and stigma sterol
5. Cucurbita maxima leaf	Thin-layer chromatography.	Cucurbita maxima leaf Thin layer chromatography. Beta-sitosterol, and Stigma sterol .
6. Cucurbita pepo leave (Cucurbita pepo (pumpkin)).	Thin-layer chromatography (TLC), (analytical and preparative) high-performance liquid chromatography, and liquid mass chromatography	phytosterol and terpenes.
7. Cucurbita ripe fruits	Flash Chromatography (FC) and HPLC	Terpenes, apolar fatty acids and lipids.
8. Edible pumpkin flowers	Gas chromatographic method.	Capric acid, Lauric acid, Myristic acid, Palmitic acid, Stearic acid, Arachidic acid, Heneicosanoic acid, and Behenic acid
9. Fruits of cucurbita pepo	colorimetric method	Flavonoid, phytosterol and steroids, triterpenoids
10.Pumpkin pulp (Cucurbita pepo)	Flame Emission spectrophotometer	Tannins

CONCLUSION:

Cucurbita maxima includes isolation techniques involving GC-MS, TLC, Flash chromatography, HPLC. Among this TLC is widely used and presence of sterols,terpenoids and flavonoids constitute the most.

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