International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Differential Chromatographic Isolation Techniques for Phytochemical and Functional Characterizations of Cucurbita Maxima Seeds

M. Dhivya[1], P. Deenadhayalan[2], D. Sandhya[3], G. Pooja[4], S. M. Prabhuroshan[5]

[1]Assistant Professor, Pannai College of Pharmacy
[2,3,4,5]Students, Pannai College of Pharmacy

ABSTRACT

Pumpkin, as a dietary plant, has been used in traditional medicine around the world. In addition, during the last decade, antidiabetic, antihypertensive, antitumor, intestinal antiparasitic, antibacterial, anti hypercholesterolemia, anti inflammatory, immunomodulatory and analgesic effects of pumpkin has been reported. There are various isolation techniques.But the present study focus the simple,reliable analytical method for isolation of bioactive compounds from pumpkin seed oil(pso).In this preliminary studies have been done for the phytochemical and chomtographic analysis studies.The study shows the results of high content of linoleic acid.secondly the circular chromatography studies reveals the presence of fatty acids. Based on this types of isolation, pumpkin seed oil have been proved to useful pharmaceutical tool.

KEYWORDS: Pumpkin Seed Oil, Phyto Chemical And Chromatographic Analysis, Fattyacids, Triglycerides, Linoleicacids, Pharamacological Activities.

AIM AND OBJECTIVE

AIM:

To do differential chromatography isolation techniques to utilize the physiochemical and functional characterization in cucurbita maxima seeds (Pumkin seeds).

OBJECTIVES:

To do the seed extract process of cucurbita maxima and obtain the pumpkin seed oil.
To collect the various review of pumpkin seeds oil and its pharmacological activity.

To collect the review of various analytical techniques involved in pumpkin.
To do chromatographic analysis by Column and Circular Chromatography in pumpkin seed oil toreportitsconsistutents and analyzing the identification of bioactive compounds.

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 diseases, including hypertension, diabetes, and cancer .

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 activities.

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.[2]

REVIEW OF LITERATURE:

PHARMACOLOGICAL ACTIVITIES:

Anticancer

Daisy.petal stated that Anticancer Activity- Anticancer activity of methanol extract of Cucurbita maxima against Ehrlich as 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.[3]

Analgesic activity

Vogel hg etal stated that 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’. [MoumitaPanda,Thin layer chromatograpic studies and in vitro free radical scavenging effects of Cucurbita maxima leaf extracts;

Antioxidant activity

Sarvesh et stated that 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

Yagi.ketal stated that Antigenotoxicspinasterol 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[4]

Diuretic Activity

Zhang etal stated that 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

Lahonetalstaed that 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[12]

Hypoglycemic activity

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

Antihyperglycemic activity

Kambojetal stated that 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. [14] 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. Adebaeyoetal stated that there were reports on broad-spectrum antimicrobial activity of pumpkin extracts. Pumpkin oil inhibits Acinetobacterbaumanii, Aeromonasveronii, 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)[15].

Anthelmintic:

Popovicetal stated that 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 andpumpkin 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.[16]

CHROMATOGRAPHIC TECHNIQUES:

Daisy.Petal 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.

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.

PA.Ekeochaetal ,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) highperformance liquid chromatography HaiderM.Kadhimetal, 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. Moumita Panda etal , stated that Cucurbita pepo (pumpkin), a Cucurbitaceae membered plant, is considered oneofthe 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) .[4]

Emir Tosinetal 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 HPLCmethod was found to be linear, accurate, precise and rugged for the quantification of the cucurbitacins studied.[4]

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 .[5]

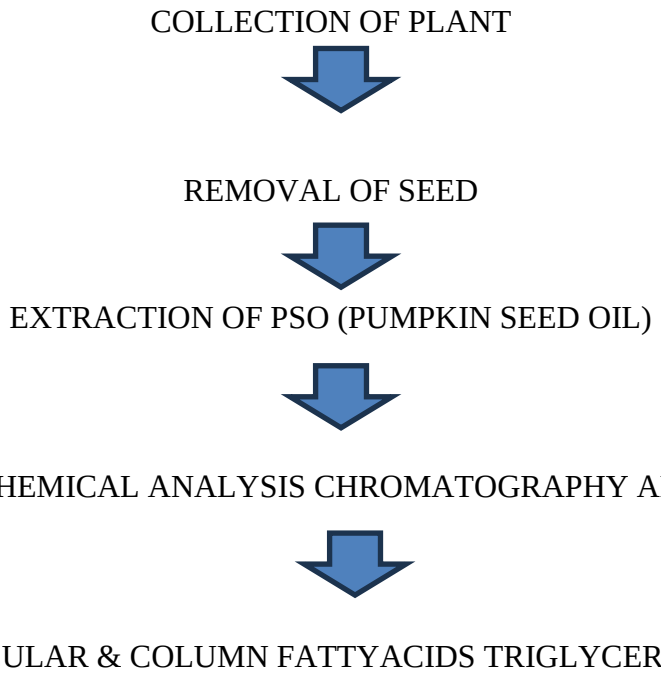
Sandeep Singh Ranaetal stated that , The edible pumpkin fowers is analysed for the physicochemical, biochemical properties, proximate analysis, antioxidant activities, anthocyanin content and fatty acid profling. Among several fatty acids' oleic acid (21%), myristic acid (15.99%) and stearic acid (15.19%) was maximum. The presence of several phytonutrients and fatty acids makes pumpkin flower a potential source of functional food in near future.[6]

Srividhya V etal stated that, Cucurbitapepo is one of the good supplement of protein, carbohydrate, minerals and fat. This coupled with high mineral content which is advantageous.

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 inoling lame emission spectrophotometer.[7]

METHODOLOGY

Though various chromatographic techniques have been studied earlier our study focuses mainly the column chromatography and circular chromatography isolation studies in pumpkin seeds suitable for the oil samples.



COLLECTION OF PLANTS:

Plant material

Pumpkin belongs to the family Cucurbitaceae. The samples were taxonomically identified by Luigi Frassineti (Tuder Green Service, Todi, Italy). It is a leafy green vegetable with medium-large flattish fruits with green-gray, moderately hard knobby skin, edible yellow/orange flesh, and a central cavity with numerous plump, whitish-yellow seeds. Three pumpkins cultivated in central Italy (Todi) and collected in autumn 2016 were selected for their uniformity of shape, weight, and color. The fresh pumpkin samples

were weighed (about 2.5 kg each), peeled, and, after manual removal of seeds, cut into small pieces (1.5 cm × 1.5 cm × 1.5 cm) and analyzed. The seeds were cleaned to remove impurities and dried at 60 ◦C for 24 h in a hot-air fan oven. After that, the seeds were reweighed until the weight was constant. The samples were stored in a dry place in the dark at room temperature.[17]

Extraction of Oil

Whole, air-dried seeds were passed through a 30 mesh screen in ahammer mill and oil was extracted by hexane for 16 hours in a largeSoxhlet apparatus. Solvent was removed from the extract by rotaryevaporation at 35°C-45°C leaving a dark green crude oil.

EXTRACTION OF PSO(PUMPKIN SEED OIL):

Pumpkin seed oil (PSO) has been known as functional food oil due to some bioactive components contained such as phenolics and tocopherols with beneficial effects to human health including antioxidants, prevention of certain cancers, and retardation of hypertension progression and alleviation of diabetes mellitus. To extract PSO from corresponding fruit seed, numerous extraction techniques either conventional like Soxhlet extraction or modern extraction systems such as ultrasound-assisted extraction supercritical extraction were optimized and developed to get maximum yields of PSO with maximum bioactive components. PSO contained tocopherols and other phenolics compounds, therefore, it has potential application in the treatment of diseases related to oxidative stress.

Due to the different price of PSO with other vegetable oils, the adulteration practice involving the substitution or addition of PSO with lower price oils such as palm oil and corn oils is possible, therefore analytical method capable of detecting the adulteration practice is available.[20]

Determination of physico-chemical properties ofPSO was needed to characterize which could be usedfurther for the authentication purposes. The chemicalcomposition and PSOs properties depend on severalfactors including varieties (cultivars) of pumpkin andregion of origins. The fatty acid (FA) composition ofPSO was significantly different among various cultivars of PSO compiles thephysicochemical characterization of pumpkin seed oil which include some constants specific for PSO have reported that the physical properties ofPSO were as follows: specific gravity of 0.9412 (at 31°C), the iodine value of 114.33 gI2/g PSO, saponificationvalue of

193.73 mg KOH/g PSO, acid value of 0.516 mgKOH/g PSO and percentage of free fatty acid of 0.2646% PSO is known to have nutritional values because itcontained some vitamins needed by the human diet have reported that the major FAs in PSO wereoleic, linoleic, and palmitic acids. In addition, PSO alsocontained δ-tocopherol, β-sitosterol, and syringic acidwas the predominant phenolic acid present in PSO. In addition, the chemical composition of PSO in terms of fatty acid composition exhibited that PSOcontained 40.58% oleic acid, 27.06% stearic acid,17.39% palmitic acid and 14.97% linoleic acid.. (2016) have noted that the polyunsaturated FA(PUFA), monounsaturated FA (MUFA) and saturatedFA (SFA) contents in PSO cultivated in southern Italywere of 48.14%, 25.54%, and 25.20%, respectively. Thehigh degree of unsaturated FA makes PSO suitable to beused as valuable drying agent, while low value of freeFA contents indicates the suitability of PSO as edibleoils.[17]

PHYTOCHEMICAL ANALYSIS:

Detection of flavonoids

Zinc hydrochloride reduction test:
Treat extract with mixture of zinc dust and concentrated hydrochloric acid. Formation of red colour indicates the presence of flavonoids.

Alkaline reagent test:
To the extract, add few drops of sodium hydroxide solution. Formation of an intense yellow colour, which turns to colorless on addition of few drops of dilute hydrochloric acid, indicates the presence of flavonoids.

Detection of tannins:
Lead acetate test:
To 5 ml of aqueous extract was treated with 1 ml of 10% lead acetate solution. Yellow colour precipitation, indicates the presence of tannins.

Vanillin hydrochloride test:
1ml of extract was added with vanillin hydrochloride. Formation of purplish red colour indicates the presence of tannins.[19]

Detection of phytosterol and flavanoids:
Small quantity of extract was dissolved in 5ml of chloroform and then subjected to the following tests.

Salkowski test:
To the above solution 1 ml chloroform and few drops of concentrated sulphuric acid was added. The test tube was shaken for few minutes. The development of red colour in chloroform layer indicates the presence of steroids.

Liebermann- Burchard reaction:
To the above solution 1ml of chloroform add few drops of concentrated sulphuric acid and 1-2 ml of aceticanhydride. Development of red colour first, then blueand finally green colour, indicates the presence ofsteroids.

Detection of saponin:
Foam test:
The extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes.Development of stable foam suggests the presence of saponins.

Froth test:
To 5 ml of test sample add few drops of sodium bicarbonate. Shake the mixture vigorously and keep it for 3 minutes. A honey comb like froth formation indicates the presence of saponins.

Detection of protein and amino acids:
Small quantity of the extract was dissolved in few ml of water and filtered. The collected filtrate was used for following tests.

Biuret test:
Filtrate was treated with 5% sodium hydroxide and few drops of 1% copper sulphate solution. Formation of violet or pink colour indicates the presence of proteins.

Ninhydrin test:
To the filtrate Ninhydrin reagent was added. Development of violet or purple colour indicates the presence of amino acids.

Detection of triterpenoids:
Libermann-Burchard test:
To extract add few drops of acetic anhydride, followed by few drops of concentrated sulphuric acid. A brown ring forms at the junction of two layers and the upper layer turn green colour, infers the presence of phytosterols and formation of deep red colour indicates the presence of triterpenoids.

Salkowski Test:
sulphuric acid was carefully added to form a layer. Formation of reddish brown coloration of the interface indicates the presence of triterpenoids.[15]

CHROMATOGRAPHICAL ANALYSIS:
Chromatography is the separation of mixtures by distribution between two or more immiscible phases. In chemical laboratories, where it is used for analysis, isolation, and purification, chromatography is the most used separation technique. It is also frequently used in the chemical process industry as a part of small and large-scale production.
There are many types of Chromatography, e.g. Adsorption Chromatography, Thin Layer Chromatography, Column Chromatography, Partition Chromatography, etc. Paper Chromatography is an example of Partition Chromatography.[14]

Chromatography Definition
Chromatography is a technique used to affect the separation of two or more dissolved solids contained within a solution in very small quantities. In Greek, the word ‘chroma’ means colour and ‘graphein’ is used to indicate writing. Initially, the technique was used for the separation of colors.
The substance that has to be segregated during the process of chromatography is known as an analyte. It basically refers to the component needed from the mixture. It is a physical process in which the solutes, that is the components of a sample mixture are segregated as a result of their differential distribution between the stationary and mobile phases.[14]

Terms used in Chromatography
Analyte: Analyte is the substance that is to be separated from the mixture during chromatography. Mobile Phase: Mobile Phase in Chromatography is the component that moves with the sample. It is either a gas or a liquid and is passed through the column where the components of the mixture are absorbed.
Stationary Phase: Stationary Phase in Chromatography is the one that doesn’t move with the sample. It is generally a porous solid that absorbs components from the mobile phase.
Eluent: Eluent refers to the fluid that enters and passes through the chromatographic column.

Elute: Elute is the fluid containing the sample that exits the chromatographic column
Elution: Elution is the process of removal or extraction of a solid by washing out it with a suitable solvent in a chromatographic column.[8]

Paper chromatography

The separation technique of chromatography is dependent on the principle of partitioning the constituent components between two phases – a Mobile Phase and a Stationary Phase. The mobile phase is used to indicate the mixture of the substances to be separated and dissolved in a liquid or a gas. The stationary phase is used to describe a porous solid matrix through which the sample present in the mobile phase percolates.

The method of chromatography is based on the fact that the dissolved substances in the same solvent may have differing solubility. The solute with a greater solubility rises faster and segregates from the mixture.[9]

The different solubility of the analyte is due to the differential affinity or the different strength of adhesion towards the mobile and stationary phases. The affinity of an analyte can be explained by two terms, the first is adsorption and the second is solubility. Adsorption is the ability to get attached to the surface of the stationary phase while solubility is the ability to dissolve in the mobile phase.[10]

Analyte having higher adsorption will slowly pass through the column while Analyte having high solubility will move faster through the column. The difference in the solubility and adsorption is due to the difference in polarity towards different phases which determines the extent of adsorption and solubility.[11] For Example, a polar analyte will adsorb better to a polar stationary phase thus moving slowly through the column, and a non-polar analyte will better dissolve in a non-polar mobile phase thus moving faster through the column.[12]

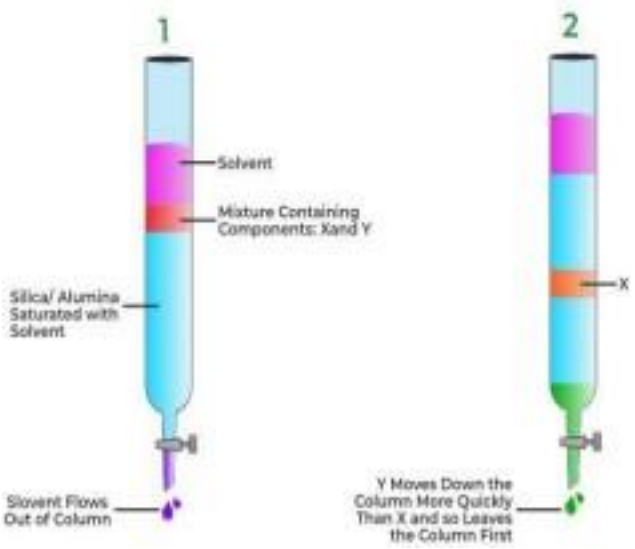
Types of Chromatography

Chromatography can be classified into different types based on the following categories based on the physical state of mobile and stationary phases. The different types of Chromatography are mentioned below:

Adsorption Chromatography
Thin Layer Chromatography
High-Performance Liquid Chromatography
Column Chromatography
Partition Chromatograp
Gel Filtration Chromatography
Liquid Chromatography
Gas Chromatography
Gas Liquid Chromatography
Adsorption Chromatography

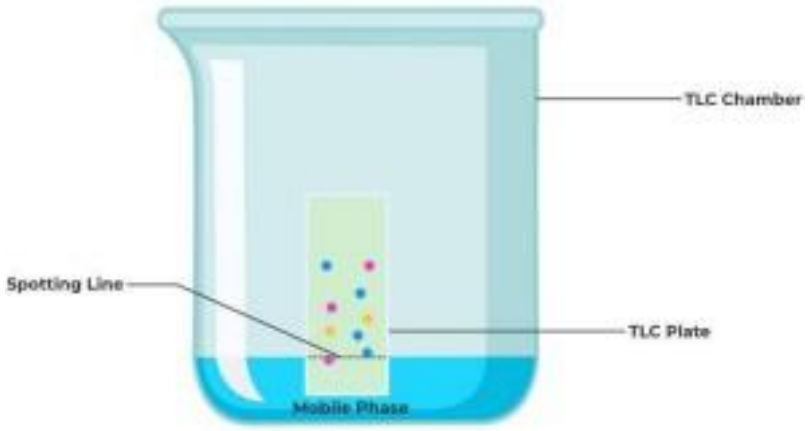
Adsorption Chromatography is based on the differential rate of adsorption of the solute to the stationary phase. In Adsorption Chromatography the mobile phase is liquid or gas in which solute is dissolved. Depending on the component's absorptivity, different solutes are adsorbed on the adsorbent to varying degrees in the adsorption chromatography process. The components with higher absorptivity are carried to a shorter distance than those with lower absorptivity in this case as well by moving a mobile phase over

a stationary phase. Adsorption Chromatography is used in the identification of carbohydrates, and fats, separation and isolation of amino acids, antibiotics, etc.[13]



Thin Layer Chromatography

Thin Layer Chromatography also called TLC Chromatography is used to separate non-volatile liquids using a thin stationary phase. As shown in the image above, the thin-layer chromatography (TLC) procedure uses a glass plate coated with a very thin layer of an adsorbent, such as silica gel or alumina, to separate the mixture of chemicals into its constituent parts.[14]



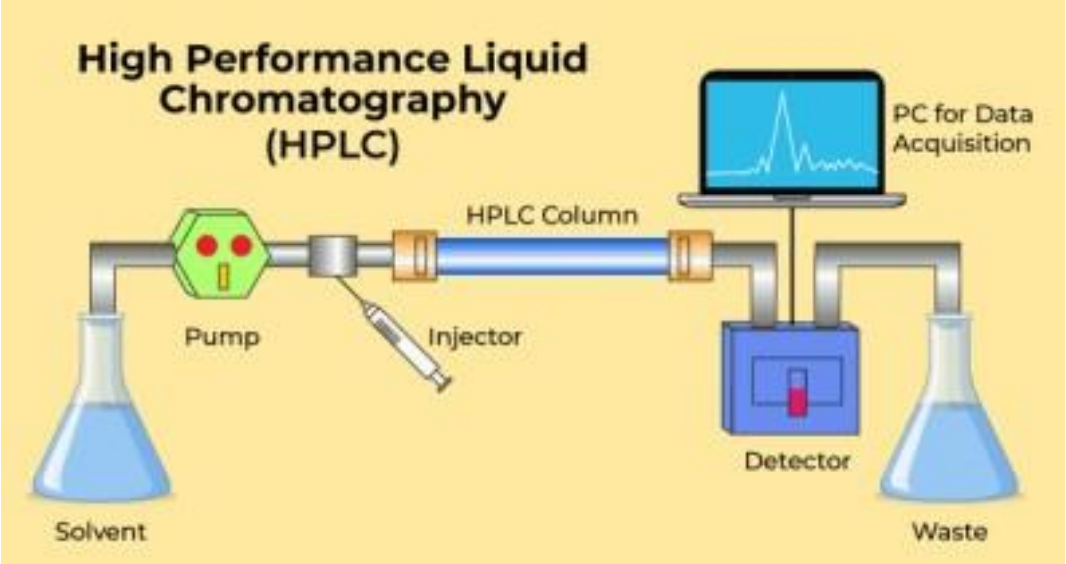
Chrome Plate is the name of the plate used in this technique. A small area of the mixture's solution is put 2 cm above one end of the plate to begin the separation process. The plate is then placed in a sealed container filled with an eluting fluid, which rises up the plate while lifting various mixture ingredients to various heights. The heights to which components rise are called spots which appear separately in a vertical arrangement. Each spot is characterized by a factor called as Retention Factor which is given by the formula mentioned below:

Retention Factor(Rf) = Distance Travelled By Sample/Distance Travelled by Solvent

High-Performance Liquid Chromatography

High-Performance Liquid Chromatography also called HPLC Chromatography is a method in analytical chemistry to separate the components of a mixture and to identify and measure their quantity. This method was introduced by M.S. Tswett who was a botanist. A sample has two types of components named analyte and matrix. It is the analyte that we want to identify and quantify and the rest of the other components of the sample are called the Matrix.[15]

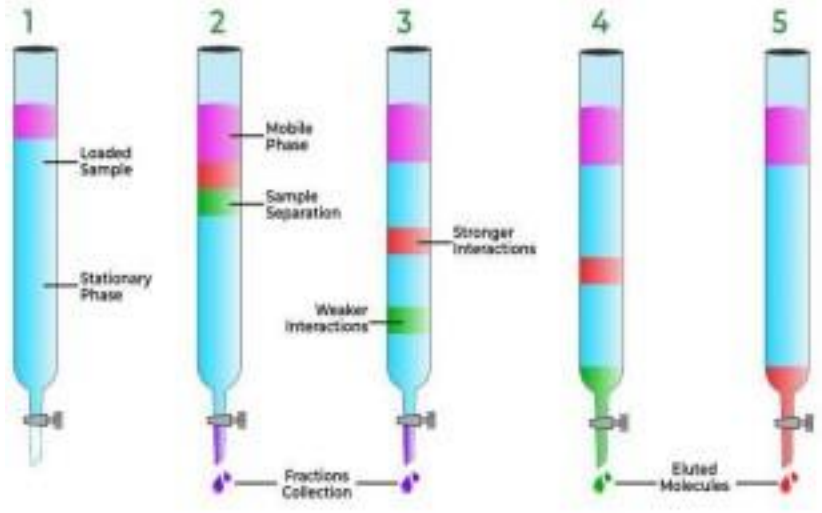
In chromatography, the sample is introduced in the mobile phase which is a liquid that passes through a solid which is usually a solid or a liquid on the solid. In HPLC, the stationary phase is in a column packed with small porous particles and the liquid passes through it with the help of a pump. The sample then passes through the column. The particles of the sample move at different rates due to the varying nature of interaction with the stationary phase. After the sample crossed the column, the substances are identified and quantified using the computer and the software attached to it.



The different types of HPLC include Normal Phase HPLC, Reverse Phase HPLC, Ion Exchange HPLC, and Size Exclusion HPLC.

HPLC is used in the analysis of drugs, synthetic polymers, environmental particles, water purification, and isolation of various valuable products.

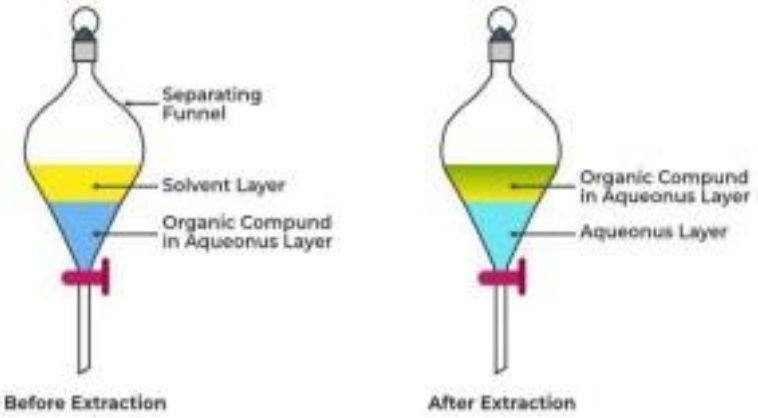
Column Chromatography



Column Chromatography is used to separate a single compound dissolved in a chemical mixture. Column Chromatography is based on the principle of differential rate of adsorption. In this method, a Column is prepared by filling the tube with a solid porous absorbent which is the stationary phase. A fluid containing the solute is passed through this column. The fluid entering the column is called eluent and in between the column solute gets adsorbed after that the fluid exits the column which is called the elute. As shown in the

figure above, column chromatography is a technique used to separate the components of a mixture using a column of appropriate adsorbent packed in a glass tube. A suitable eluant is produced to flow gently down the column in drop by drop manner after the mixture is placed on top of it.[16] The components are separated based on how much of each component has been adsorbed to the wall of the adsorbent column. The element with the greatest absorptivity is kept at the top while the other elements flow downward to various heights in accordance. Column Chromatography is a low-cost technique used to isolate active ingredients from a compound, remove impurities, and in drug estimation.

Paper Chromatography (Partition Chromatography)



Partition Chromatography was introduced by Archer Martin and Richard Laurence. Partition Chromatography is used to separate the component from liquid-liquid phases. In Partition Chromatography, the retention factor is given by the distance travelled by the component from the mobile phase to the stationary phase and then back to the mobile phase. In Partition Chromatography one phase is polar and the other is non-polar. The difference in polarity leads to the separation of components.[17]
A continuous differential partitioning of mixture components into a stationary phase and a mobile phase occurs in this process. Paper chromatography is a good example of partition chromatography. The stationary phase in this procedure is chromatography paper, which is suspended in a combination of solvents acting as the mobile phase. The mixture to be separated is placed at an area at the bottom of the chromatographic paper, and as the solvent rises up the paper, the components are carried to varying degrees depending on how well they adhere to the paper. As a result, the components are divided at various heights.

Gel Filtration Chromatography

One of the types of Partition Chromatography is Gel Filtration Chromatography. Gel Filtration Chromatography is based on the principle that molecules are partitioned between the stationary phase and a mobile phase that consists of a porous matrix whose porosity is of defined nature. The column of such a matrix has two measurable liquid volumes named external and internal volumes. The molecules greater than the size of pores of the stationary phase will exclude from internal volume. The molecules whose size is smaller than the pores of the stationary phase make equilibrium with external and internal volume and migrate slowly. These smaller particles exclude the volume larger than the external volume.[18] Hence, in Gel Filtration Chromatography, the particles are separated in decreasing order of their molecular size. Gel Filtration Chromatography is used to separate proteins and peptides, cells and viruses, and other biomolecules.

Liquid Chromatography

Liquid Chromatography is a chromatographic technique in which the mobile phase is liquid. The solute is dissolved in the liquid mobile phase which is then passed through the column surrounded by the stationary phase or the plane over the stationary phase. The solute dissolved in the liquid phase gets adsorbed to the stationary phase depending on its interaction with the stationary phase. Liquid Chromatography is an essential tool when the mixture that is to be separated is colored. Depending on the components of chromatography Liquid Chromatography is of four types namely, Reversed Phase, Size Exclusion, Normal Phase, and Ion Exchange Chromatography. Liquid Chromatography is used in testing food quality, forensic labs, testing ink samples, and environmental analysis. It is an easily available, relatively inexpensive technique and performed manually with better control.[19]

Gas Chromatography

Gas Chromatography is a technique used to separate volatile components in the gas phase. Gas Chromatography is a chromatographic technique that uses an inert gas as the mobile phase. When the stationary phase is solid in Gas Chromatography then it is called Gas-Solid Chromatography while when a liquid on an inert layer is used as a stationary phase then it is called Liquid-Gas Chromatography. A Gas Chromatographer consists of a sample injection unit, a column, and a detector. The sample to be analyzed is injected into the sample injection system which heats up the sample and vaporizes it. After the sample heats up it moves up along with the gas mobile phase through the column which is separated by the stationary phase and the final compound is analyzed by the detector. The technique of Gas Chromatography was introduced by Mikhail SemenovichTsvett. Gas Chromatography is extensively used for the analysis of volatile mixtures, in analyzing air samples to analyze air quality, in the pharmaceutical industry, and in analyzing environmental toxins.[20]

Gas-Liquid Chromatography

We know that every Chromatography involves two types of phases named mobile phase and stationary phase. In Gas-Liquid Chromatography, the mobile is a gas like helium and the stationary phase is a liquid which is of high boiling point and is absorbed into a solid. The velocity of movement of a compound in the chromatography machine depends on the time it spent with the gas i.e. the mobile phase.

The working of Gas-Liquid Chromatography can be understood in the following steps:

Step 1: First the sample is injected using a syringe in a temperature controlled oven. Here, the sample boils and moves into the column in gaseous form with the help of helium gas i.e. mobile phase.

Step 2: The column is packed with porous rock coated with a waxy polymer.

Step 3: The temperature of the column ranges between 50C to 250C. With the proceeding of analysis, the temperature starts rising.

Step 4: Inside the column, the sample may condense, it may dissolve in the liquid, or may remain in the gas. In general, the substance partitions itself between the gaseous mobile phase and the stationary liquid phase. Any molecule of the sample spends some of its time with the liquid stationary phase and some with the gaseous mobile phase.

Column chromatography:

Column chromatography separates substances based on differential adsorption of compounds to the

adsorbent as the compounds move through the column at different rates which allows them to get separated in fractions. This technique can be used on a small scale as well as large scale to purify materials that can be used in future experiments. This method is a type of adsorption chromatography technique.[2]

When the mobile phase along with the mixture that needs to be separated is introduced from the top of the column, the movement of the individual components of the mixture is at different rates. The components with lower adsorption and affinity to the stationary phase travel faster when compared to the greater adsorption and affinity with the stationary phase. The components that move fast are removed first whereas the components that move slowly are eluted out last.
The adsorption of solute molecules to the column occurs in a reversible manner. The rate of the movement of the components is expressed as:

Rf = the distance travelled by solute/ the distance travelled by the solvent.

The stationary phase is made wet with the help of solvent as the upper level of the mobile phase and the stationary phase should match. The mobile phase or eluent is either solvent or a mixture of solvents. In the first step the compound mixture that needs to be separated, is added from the top of the column without disturbing the top level. The tap is turned on and the adsorption process on the surface of silica begins. Without disturbing the stationary phase solvent mixture is added slowly by touching the sides of the glass column. The solvent is added throughout the experiment as per the requirement.

The tap is turned on to initiate the movement of compounds in the mixture. The movement is based on the polarity of molecules in the sample. The non-polar components move at a greater speed when compared to the polar components.

For example, a compound mixture consists of three different compounds viz red, blue, green then their order based on polarity will be as follows blue>red>green As the polarity of the green compound is less, it will move first. When it arrives at the end of the column it is collected in a clean test tube.
After this, the red compound is collected and at last blue compound is collected. All these are collected in separate test tubes.[3]

COLUMN CHROMATOGRAPHY BY TRIGLYCERIDES

TRIGLYCERIDES:

The TG portion of refined oil and each fraction were separatedby a column chromatographic method as follows (31): Silica gel (ResearchSpecialties Company, 200 South Garrad Boulevard, Richmond, California)was dried in the oven at 200°C for 2 hours in a sealable container.After drying, the gel was allowed to stand in a desiccator at roomtemperature for 30 minutes. Dried silica gel was weighed into a 400 mlbeaker, 5% distilled water (w/w) was added, and thoroughly mixed. Then,30 g of hydrated silica gel were weighed into a 250 ml beaker. Thecolumn was filled about half full with petroleum ether, and hydratedsilica gel was added to the column with tapping to dispel air bubblesand to insure uniform packing of the column.When the level of the petroleum ether fell to 2 cm above the topof the silica gel, 5.0 ± 0.01 g of oil were added to the column in 20 mlof benzene. The eluate was collected in a 400 ml beaker. When thebenzene level fell to 2 cm above the silica gel, the san^ile beaker rinsed with 10 ml
of benzene, which was added to the column. Thiswashing was repeated two times more. A 250 ml separatory funnel wasconnected to the column, and 250 ml of benzene were allowed to flowthrough the column. The benzene fraction which contained only the TGs(not the mono- or diglycerides) was collected. Most of the benzene was removed on a steam bath under a hood. The final benzene vapors wereremoved from the TG portion by heating in a vacuum oven at 70"C untila constant weight was obtained. TGs were stored at 4 C until preparation of methyl esters.[6]

CIRCULAR CHROMATOGRAPHY:

Chromatography technique that uses paper sheets or strips as the adsorbent being the stationary phase through which a solution is made to pass is called paper chromatography. It is an inexpensive method of separating dissolved chemical substances by their different migration rates across the sheets of paper. It is a powerful analytical tool that uses very small quantities of material.[7]

The principle involved can be partition chromatography or adsorption chromatography. Partition chromatography because the substances are partitioned or distributed between liquid phases. The two phases are water held in pores of the filter paper and the other phase is a mobile phase which passes through the paper. When the mobile phase moves, the separation of the mixture takes place. The compounds in the mixture separate themselves based on the differences in their affinity towards stationary and mobile phase solvents under the capillary action of pores in the paper. Adsorption chromatography between solid and liquid phases, wherein the solid surface of the paper is the stationary phase and the liquid phase is the mobile phase.[19]

CIRCULAR PAPER CHROMATOGRAPHY BY FATTY ACDS

FATTYACIDS:

A simple and rapid method for the separation and identification of fatty acids and glycerides has been developed by using circular paper chromatography. Petroleum hydrocarbon, dodecylbenzene, and tetralin were used as the stationary solvents. As the developing solvents, 90% acetic acid and methanol-acetic acid, both saturated with a stationary solvent, were suitable for the separations of fatty acids and of glycerides respectively. The fatty acids were detected on paper by successive treatment with 0.5% lead acetate, water, and ammonium sulfide solution. The selective detection of unsaturated acids by spraying with a 2% solution of iodine in ethanol was also applied. The separation of various critical partners of fatty acids was achieved by the method of double development, involving the first low-temperature development with petroleum ether under dry ice and the second reversed phase development with the usual solvent systems at room temperature. The unsaturated glycerides were chromatographed as their mercuric acetate addition compounds, and were detected by spraying with a 0.2% solution of diphenylcarbazone in ethanol or by soaking in a Sudan black solution. The clear-cut separations of fatty acids and glycerides into their homologs can be attained by these circular chromatographic techniques, since the separation of unsaturated triglycerides mainly depends on their unsaturation and that of mono-, di-, and triglycerides mainly depends on the number of their free hydroxylgroups.[15]

RESULTS &DISCUSSION:

The results of pumpkin seed oil by column chromaography showed a higher content of oleic acid than linoleic acid (41.4% vs. 37.0%) C. maxima seed oil were linoleic acid (47.45%), followed byoleic (25.54%) and palmitic (17.58%) acids.pumpkin (C. maxiseed oil contained a high amount of oleic acid, 40.58%, while linoleic acid was 14.97%.

By circular chromatography it seems to be a successful method of analysis of various fats, fatty acids,.

The RF value was found to be 0.58 andIt was confirmed to be of great value as a simple, rapid, and practical method for analysis.

TABLE.1.PHYSIOCHEMICAL PROPERTIES OF SOXHLET EXTRACTION

Oil Stability Index (h)	8.56±1.05c
State at room temperature	Liquid

TABLE.2. FATTY ACIDS OF SOXHLET EXTRACTION

COMOPOSITION FATTY ACID PROFILE(%)	SOXHLET EXTRACTION
Palmitic Acid (C16:0)	19.57±1.28a
Palmitoleic Acid (C16:1)	0.18±0.01b
Stearic Acid (C18:0)	9.32±0.56a
Oleic Acid (C18:1)	25.51±1.12a
Linoleic Acid (C18:2)	44.39±2.18b
α -Linolenic Acid (C18:3)	0.48±0.01a

Arachidic Acid (C20:0)	0.16±0.01a
Behenic Acid (C22:0)	0.22±0.01a
Lignoceric Acid (C24:0)	0.17±0.01a
SFA	29.44±1.05a
USFA	70.56±1.79a

PARAMETRS	SOXHLET EXTRACTION
Oil yield (%)	38.03±1.09a
Refractive index (40°C)	1.46±0.01a
Specific gravity (25 °C)	0.91± 0.01a
Iodine value (g I2/100 g oil)	105.33±0.58b
Peroxide value (meq O2/kg oil)	3.88± 0.17a
Acid value (mg KOH/g oil)	1.35± 0.04a
Saponification value (mg KOH/g oil)	176.13±2.46ab
Unsaponifiable matter (%)	0.88±0.02a
R value (k232/k270)	0.91± 0.01a
Color (Lovibond) Y	47.21±0.09a
R	3.7 ± 0.15b

TABLE.3. TRIGLYCERIDES OF SOXHLET EXTRACTION

COMPOSITION TAG profile (%)	SOXHLET EXTRACTION
LLL	19.04± 0.02a
OLL	17.03±0.02c
PLL	17.01±0.03a
LOO	11.47±0.01a
OOO	8.46±0.02b
PLO	8.49±0.02b
SLL	5.41±0.01a
SOL	4.81±0.01a
POO	2.48±0.01a
OLLn	2.51±0.02b
LLLn	1.93± 0.02b
PLLn	0.84±0.01a
LLnLn	0.84±0.01a

CONCLUSION:

The chromatographic analytical studies reveals the presence of triglycrides such as oleicacid,linoleic acid and palmitic acid in column chromatography and once again the fatty acids by circular chromatography.

The phytochemical studies of pumpkin seed oil was done.

Based on this studies composition, pumpkin seed oil cabe employed in antiasthmatic, anti-inflammatory, antihyperlypidemic and even in dermaologicalsutdies due to its high contents.

REFERENCES:

1. Daisy.P, WinfanCelebs.J, PonNivedha.R, Bioinformatics Centre; April-June 2014 HPLC,GC MS and in-silico analysis of Cucurbita maxima methanolic extract for its activity against Prostatecancer;pp 500-505,vol 6.
2. Alhassan, Siddig Ahmed and Salwa ME Khogali, the National Ribat University, Medicinal & Analytical chemistry International Journal gas chromatography mass spectrophotometry Analysis report about Pumpkin flowers med.
3. PA.Ekeocha, CO.Ezeh, JV.Anyam,KC. Onyekwelu; Isolation, Structural Education and therapeutic potentials of root of Cucurbitapepo.
4. Haider M.Kadhim1,Maha N.Hamad,Yasir M.Kadhim2; Isolation and identification of two Cucurbita B and E and Detection of phytosterols in Cucurbitapepo ;June 24,2020; revised: July 25,2020; accepted: August 20,2020.
5. MoumitaPanda,Thin layer chromatograpic studies and in vitro free radical scavenging effects ofCucurbita maxima leaf extracts; January 2011.
6. HaiderM.Kadhim, Isolation and identification of two Cucurbita find B and E and detection of phytosterols in CucurbitapepoL.var.pepo(pumpkin) Leaves extract; September 2020.369-373.
7. EmirTosin and AhmetBaysar; Isolation and purification of cucurbitacin D and I from EcballiumElaterium(L).A.Rich Fruit juice; December 2019.
8. Sandeep Singh Rana , PayelGhosh; Physicochemical, nutritional, bioactive compounds and fatty acid profiling of pumpkin flower(Cucurbita maxima), as a potential functional food; September 18,2020/ Accepted: December 28,2020/published online: January 25,2021.
9. Srividhya V, SengottuvelThangavel, GopalaSatheeskumar K, Kanupriya J, AriharaSivakumar G; Research article antioxidant potential and phytochemical analysis of fruit extract of Cucurbita pepo, Volume 6 issue-3-2019.
10. Adebayo. O.R, Farombi A.G, Oyekanmi A.M; Mineral and anti nutrient evaluation of pumpkin pulp(Cucurbitapepo): Volume 4, issue 5(may-jun,2023) 11.Kamboj, V.P. Herbal Medicine. Current Science 2000; 78:35-51.
11. MacGibbon DB, Mann JD. Inhibition of animal and pathogenic fungal proteases by phloem exudate from pumpkin fruits (Cucurbitaceae). Journal of the Science of Food and Agriculture. 1986 Jun;37(6):515-22.
12. Popovic M., On growing squash and pumpkin (Cucurbita ap.) in yougoslavia, Savremena, Poljoprivreda, 1971, 11; 59-71.
13. Kleinig, H., Filament formation in vitro of a sieve tube protein from Cucurbita maxima and Cucurbitapepo. Planta (Berlin), 1975, 127, 163–170.
14. LahonLC,KhanikorHN,AhmadN,Gogoi AR, Preliminary and pharmacological and anticestodal screening of Cucurbitamaxima.Indian journal of pharmacology.1978 Oct 1;10(4):315.
15. Saha P,UK M,PK H,NaskarS,KunduS,BalaA,KarB.Anticancer activity of methanol extract ofCucurbita maxima against Ehrlich ascites carcinoma.

16. ZhangY,Yao H. Study on effect of hypoglycemia of different type pumpkin.Journal of Chinese food science.2002;23:118-20.
17. SarveshDharDubey(2012) Overview on Cucurbita maxima. International journal of phytopharmacy. Vol.2(3), pp.68-71, may-jun 2012.
18. Yagi,K., 1987.Lipid peroxides and human disease.Chemistry and physics of lipids 45,337- 341.
19. Vogel HG. Drug discovery and evaluation.2nd ed. Germany: Springer Verlag Berlin Heidelberg 2002;948-1051.