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RESEARCH ARTICLE

GCMS AND TLC ANALYSIS OF ETHANOLIC EXTRACT OF SEED MOMORDICA CHARANTIA

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Abstract

To perform the TLC and GCMS analysis of ethanolic extract of seed Momordica charantia. The seeds ofMomardicacharantia was extracted with ethanol and tested for TLC and GCMS by standard procedure as per the guidelines of WHO.The phytochemical and TLC studies showed the presence of alkaloids, cardiac glycosides, phenols, steroids, terpenoids and proteins.The GCMS analysis of seed Momardicacharantiashowed five major compounds were found to be n-hexadeconic acid, 9,12-Octadecadinoic acid,Octadecanoic acid,Hexadecanoic acid-2-hydroxy-1-hydroxy methyl ethyl ester and Gamma-sitosterol.The Therapeutic activity may be due to the major compounds identified by GCMS and further have to be evaluated.Mostly cucurbitaceae plant possesses the highest nutritive value and also a good source of carbohydrates, proteins, fibers, vitamins, and minerals. Fruits are composed of sufficient of water,protein and lipids respectively.In addition to this MC seeds can represent a good source of lipids, such as polyunsaturated fatty acids and they are among the few foods containing conjugated linolenic acid, being as eleostearic acid. The essential oil, obtained from drought seeds, contains sesquiterpenes, phenylpropanoids and monoterpenes. Other bioactive compounds, such as tocopherols and polyphenols have been reported in general. The pericarp, the aril, the stem and the leaves of the plant are also a good source of phenolic compounds, which can be useful to protect from oxidative damage by acting directly on reactive oxygen species and to induce endogenous defense systems .

Introduction:-

India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative system of health namely Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy. These systems are rightfully existed side by side with allopathy are not in domain of obscurity.[1]A boon towards the Herbal drugs and their procurement is grown faster in both developing and developed countries which is mainly due to their natural origin, low cost, easy availability and lesser side effects, still now the pharmacopoeial standards for herbal raw materials or finished products was not found.[2]On behalf of this World Health Organization has set a certain guidelines for the assessment of safety, efficacy and quality of herbal medicines.[3]. Momordica charantia is rich in nutrients like

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thiamine, beta carotene, foliate, riboflavin and minerals like calcium, iron, phosphorous, manganese, potassium, magnesium, zinc and dietary fibres. Regular intake of bitter gourd juice boosts the immunity and effective in early stage of cholera and other types of diarrhoea. Life threatening diseases such as hypertension, diabetes mellitus have been treated with juice of Momordica charantia.[4-6]

Materials And Methods:-

The fruit of Momordica charantia were collected from Thyagaraja Nagar, Chennai, Tamil Nadu, India during November 2019. The plant material was identified by professor Dr. J. Jayaraman, Ph. D. Director, plant Anatomy Research center, west Tambaram, Chennai. A voucher specimen (PARC/2019/4177) was submitted at C. L. Metha College of Pharmacy, Chennai- 97. The seeds were separated, shade dried, Pulverized and extracted by hot percolation (Soxhlet) method by using ethanol as solvent. The extract obtained was tested for phytochemical and GCMS studies [7]

Thin Layer Chromatographic Study:

Plant extract was subjected to thin layer chromatography (TLC) as per conventional method using silica gel as adsorbent with a appropriate measurement the TLC plate were cut using TLC cutter markings in the plate were made with a pencil.Glass capillary tubes were used to spot the extract in TLC plates. The solvent system was tested for the separation of bioactive components. In the TLC chamber the solvent system viz butanol: acetic acid: water (4:1:2) were used. After pre-saturation with mobile phase for 30 min the plates were kept inside the chamber and the elution was performed using above mentioned solvent systems. After completion of the elution the plates were dried and subjected to visualized under UV chamber and sprayed using iodine spray reagent.[8] R_f values determined by using following formula:

R_f = Distance travelled by the solute / Distance travelled by the solvent

GAS CHROMATOGRAPHY:

Procedure:

Sample Preparation

The Seed extracts was given for GCMS studies by standard procedure and then analysed and interpreted.

Sample Injection

A sample port is used for placing the sample at the head of the column. Modern injection techniques often employed by the use of heated sample ports through which the sample is made to be injected and vaporized . A calibrated micro syringe is used to deliver a microlitre range of samplethrough a rubber septum and into the vaporization chamber. Most separations technique involved require only a small fraction of thesample and also a sample plitter device is used to disguard out the excess sample. Commercial gas chromatographs often allow for both split and splitless injections when it is placed between packed columns and capillary columns. The vaporization chamber is typically heated at 50 °Cthe sample is subsequently mixed with the carrier gas and passed into the column.

Carrier Gas

The carrier gas plays an important rolein the GC technique. Carrier gas used must be dry, free of oxygen and chemically inert. Helium is most commonly used because it is safer but comparable to hydrogen it has a larger flow rates and is compatible with many detectors. Nitrogen, argon, and hydrogen are also used depending uponthe detectors used. Both hydrogen and helium, which are commonly used on most of the detectors such as Flame Ionization (FID), thermal conductivity (TCD) and Electron capture (ECD), which provide a shorter analysis time and lower elution technique of the sample due to its higher flow rates and low molecular weight. For instance, hydrogen or helium used as the carrier gas gives the highest sensitivity in thermal conductivity detector, because the organic vapor and hydrogen/helium is greater than of any other carrier gas. Other detectors such as mass spectroscopy, uses nitrogen or argon which has a much better advantage than hydrogen or helium due to their higher molecular weights,which improves vacuum pump efficiency.

All carrier gasses are available in pressurized tanks and pressure regulators, gauges and flow meters are used to check and control the flow rate of the gas. Most gas supplies used should fall between purity range and contain a low level of oxygen and total hydrocarbons in the tank. The carrier gas system possess a molecular sieve to remove water and other impurities. Traps are another option to keep the system pure and optimum to remove the traces of water and other contaminants. A two-stage pressure regulation is required to minimize the pressure surges and

monitor the flow rate of the gas.This indicates that different gas type use a various type of regulator. The carrier gas is preheated and filtered with a molecular sieve to remove impurities and water,and then introduced to the vaporization chamber. A carrier gas is typically required in GC system to flow through the injector and push the gaseous components of the sample onto the GC column, which is then directed to the detector.

Column Oven

The thermostatted oven serves to control the temperature of the column.The oven can be operated in isothermal programming or temperature programming manner. In isothermal programming, the temperature of the column is kept constant throughout the entire separation process. The optimumtemperature is maintained for isothermal operation, howeverisothermal programming works best only if the boiling point range of the sample is narrow. If a low isothermal column temperature is used with a wide range of boiling point, then the low boiling fractions are well resolved but the high boiling fractions are slow to elute with extensive band broadening. If the temperature is increased closer to the boiling points of the higher boiling components, the higher boiling components elute as sharp peaks but the lower boiling components elute so quickly without any peaks and separation will not takes place.

In the temperature programming method, the column temperature is either increased continuously or in between the intervals as the separation progresses. This method is well suited to separating a mixture with a broad boiling point range. The analysis begins at a low temperature to resolve the low boiling components and increases during the separation to resolve the less volatile, high boiling components of the sample.

Open Tubular Columns And Packed Columns

Open tubular columns, which are also known as capillary columns, come in two basic forms. The first is a wall-coated open tubularcolumn and the second type is a support-coated open tubular column. WCOT columns are capillary tubes have a thin layer of the stationary phase coated along the column walls. In SCOT columns, the column walls are first coated with a thin layerof adsorbent solid, such as diatomaceous earth, a material which consists of single-celled, sea-plant skeletons. The adsorbent solid is then treated with the liquid stationary phase. While SCOT columns as a capacity to hold greater volume of stationary phase than a WCOT column due to its greater sample capacity.

Most modern WCOT columns are made of glass, but stainless steel, aluminium, copper and plastics have also been used. Each material has its own separation technique merits depending upon its application. Glass WCOT columns have the distinct advantage of chemical etching, which is usually achieved by gaseous or concentrated hydrochloric acid treatment. The etching process gives the glass a rough surface and allows the bonded stationary phase to adhere more compactly to the column surface.

One of the most popular types of capillary columns is a special WCOT column called the fused-silica wall-coated (FSWC) open tubular column. The walls of the fused-silica columns are drawn from purified silica containing minimal metal oxides. These columns are much thinner than glass columns, with diameters range between 0.1 mm and lengths as long as 100 m. To protect the column, a polyimide coating is applied to the outside of the tubing and bent into coils to fit inside the thermostatted oven of the gas chromatography unit. The FSWC columns are commercially available and currently replacing older columns due to increased chemical inertness, greater column efficiency and smaller sampling size requirements. It is possible to achieve up to 400,000 theoretical plates with a 100 m WCOT column.

Packed columns are made of a glass or metal tubing which is densely packed with a solid support like diatomaceous earth. Due to the difficulty of packing, these types of columns have a larger diameter than open tubular columns and have a limited range of length. As a result, packed columns can only achieve about 50% of the efficient separation in comparison with WCOT column. Furthermore, the diatomaceous earth packing is deactivated over time due to the semi-permanent adsorption of impurities within the column. In contrast, FSWC open tubular columns are manufactured to be virtually free of these adsorption problems.

Detection Systems

The detector is the device located at the end of the column which provides a quantitative measurement of the components of the mixture as they elute in combining with the carrier gas. In instance, any property of the gaseous mixture that is different from the carrier gas can be used as a detection method. These detection properties fall into two categories: bulk properties and specific properties. Bulk properties, which are properties that both the carrier gas

and analyte possess but to different degrees. Specific properties, such as detectors that measure nitrogen-phosphorous content, have limited applications but as a increased sensitivity.

Each detector has two main parts that is when used together they serve as transducers to convert the detected property into an electrical signal that is recorded as a chromatogram. The first part of the detector is the sensor which is placed as close to the column.The second is the electronic equipment used to digitalize the analogy signal so that a computer may analyse the acquired chromatogram. The sooner the analogy signal is converted into a digital signal, the greater the signal-to-noise ratio increases, as analogy signal are easily susceptible to many types of interferences.

EXTRACT	SOLVENT SYSTEM	NO OF SPOTS	Rf VALUE
MC SEED	Butanol: Acetic acid: water(4:1:2)	9	0.16, 0.33, 0.46, 0.5, 0.56, 0.66, 0.73, 0.8, 0.93

An ideal GC detector is distinguished by several characteristics. The first requirement is adequate sensitivity which provides a high-resolution signal for all components in the mixture. This is ideal that as soon as a sample would approach zero volume and the detector would need infinite sensitivity to detect it. In modern instruments, the sensitivities of the detectors are in the range of 10^{-8} to 10^{-15} g of solute per second. Furthermore, the quantity of sample must be reproducible and enough amount of sample to be injected in column to distort peaks.An ideal column will also be chemically inert and should not alter the sample. Optimized columns will be able to withstand temperatures in the range of -200 °C to at least 400 °C. In addition, such a column would have a short linear response time that is independent of flow rate and extends for several orders of magnitude. Moreover, the detector should be reliable, predictable and easy to operate [10-12].

Result And Discussion:-

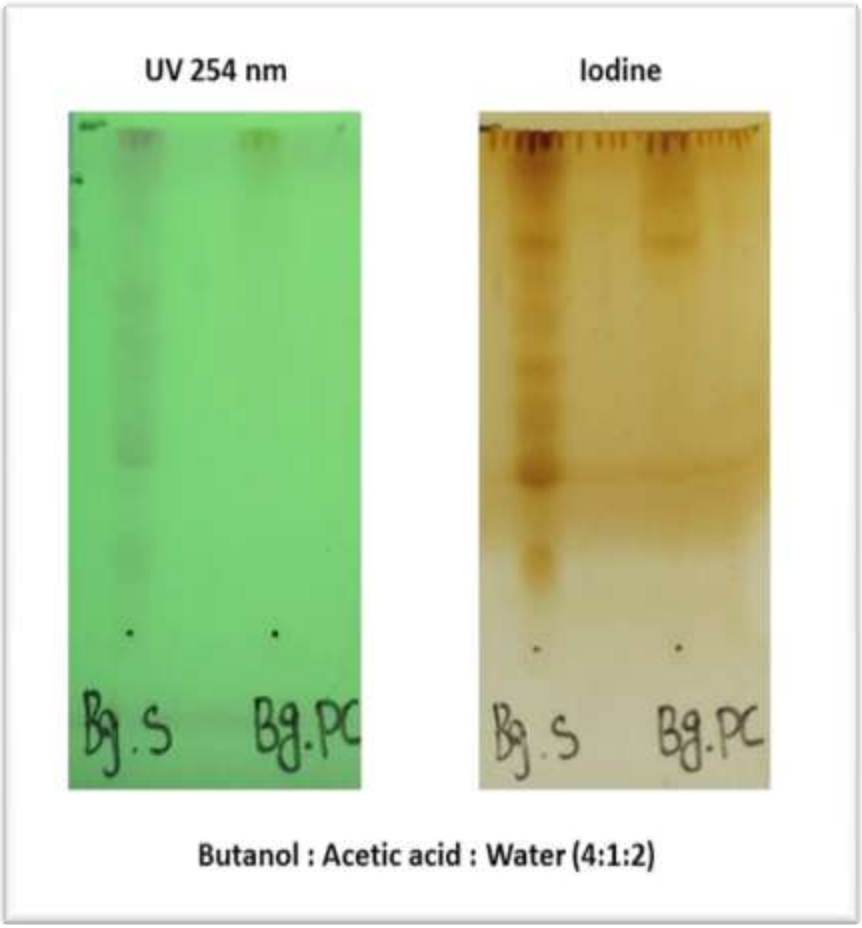
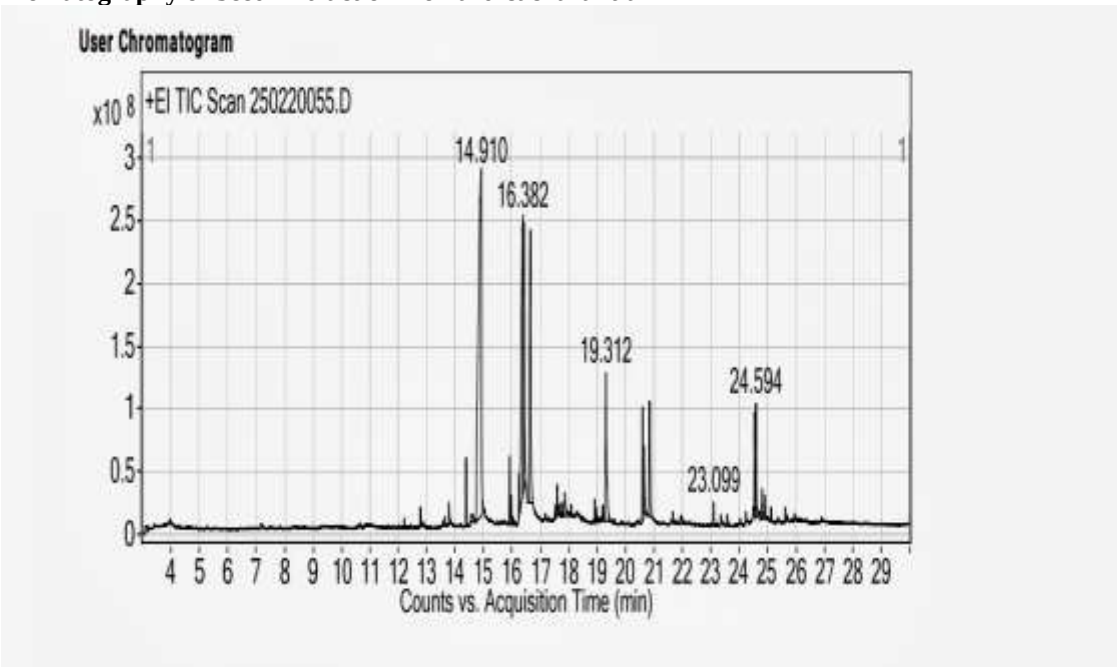


Table No. 1:- Thin Layer Chromatography Rf value.

Gas Chromatography of Seed Extract of Momardicacharantia



Graph No. 1:-

Table No. 2:- Major Compounds Present in Seed of Momardicacharantia.

Retention Time	Name of the compound	Area	Formula	Mass	Area
14.91	n-Hexadecanoic acid	1885539527	$C_{19}H_{32}O_2$	256.2	31.66
16.38	9,12-Octadecadienoic acid(Z,Z)	1007995273	$C_{18}H_{32}O_2$	280.2	16.92
16.65	Octadecanoic acid	621753465	$C_{18}H_{32}O_2$	284.3	10.44
19.31	Hexadecanoic acid, 2-hydroxy-1(hydroxy methyl) ethyl ester	320811410	$C_{19}H_{38}O_4$	330.3	5.39
24.59	Gamma sitosterol	189041597	$C_{29}H_{500}$	414.4	3.17

The solvent used for extraction is ethanol. The ethanolic extract of Momardicacharantia pericarp and seed were measured and the qualitative phytochemical analysis of Momordica charantia ethanolic extract of pericarp and seed were performed. The pericarp showed the presence of alkaloids, saponins, tannins, flavonoids, phenols, steroids, terpenoids, quinones and proteins. The seed showed the presence of alkaloids, cardiac glycosides, phenols, steroids, terpenoids and proteins.TLC analysis was performed for both extracts using mobile phase butanol: acetic acid: water (4:1:2). Pericarp showed 3 spots and seed showed 9 spots with Rf value.The GCMS analysis was performed for seed of Momardicacharantia. The compounds showed higher peak area and retention time were taken and around 26 compounds were identified.The compounds of higher peak area and retention time were given as follows with 910-n-hexadeconic acid, 16.382-9,12-Octadecadinoic acid, 19.312-Hexadecanoic acid-2-hydroxy-1-hydroxy methyl ethyl ester, 24.594-Gamma-sitosterol.

Discussion:-

The well-known common medicinal Momardicacharantia plant parts pericarp and seed were selected for study purpose. The plant has vast review in research and proven a vast therapeutic activities. The universal solvent ethanol

was used for extraction. The yield of ethanolic extract of Momardicacharantiapericarp and seed were quantified. The qualitative phytochemical analysis of Momordica charantia ethanolic extract of pericarp and seed showed the presence of alkaloids, phenols, steroids, terpenoids and. The chromatographic analysis was performed for both extracts using mobile phase butanol: acetic acid: water (4:1:2) in TLC and the major Rf value spots are 0.8,0.9 and 0.96.The GCMS analysis was performed for seed of Momardicacharantiashowed Four major compounds were found to be n-hexadeconic acid, 9,12-Octadecadinoic acid, Hexadecanoic acid-2-hydroxy-1-hydroxy methyl ethyl ester and Gamma-sitosterol.The above results showed Momardicacharantia seed was better therapeutic activity than pericarp in the treatment of colon cancer. The Therapeutic activity may be due to the major compounds identified by GCMS and further have to be evaluated.

Conclusion:-

Natural Product research was giving enormous opportunities in Drug Discovery which help in better compatibility with the human body, lesser side effects. The medicinal plant constituents do wonders in the new invention of Drug molecules, the plant Momardicacharantia also such a common plant which was used traditionally for longer period and had proven diabetes and cancer activity. This above phytochemical, invitro and GCMS studies of plant part seed and pericarp of MC will give new incentive to the natural system of medicine in the treatment of colon cancer. The Plant can be further tested for preclinical and clinical studies in the treatment of colon cancer.

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Conflict Of Interest

No conflict of Interest.

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