TLC of aqueous extract of Carica Papaya

Abstract: The tree of Carica papaya is always with soft main trunk and it is tufted with leaves that are at the top. Its fruits differ in shape, size, taste and colour. Papaya is cultivated in almost all of the tropical countries and it is used as a medicine. The Quecertin flavonoid is a plant pigment that is found in many plants and food, it is used as a standard in the TLC[WU1] of aqueous of Carica papaya extraction. The main thing [WU2] of this experiment is to optimize the TLC protocol of aqueous extract of Carica papaya by using the right mobile solvent that will give the best resolution. The separation depends on the relative affinity of compounds towards stationary phase. The compounds under the influence of the mobile phase (driven by capillary action) travel over the surface of the stationary phase. During this movement, the compounds with high affinity to stationary phase travels slowly while the others travel faster. Thus, separation of components is achieved.[WU3]

Keywords: Carica papaya, Quecertin Flavonoid, thin- layer chromatography.

Introduction [WU4]

Carica papaya is a plant that is commonly known as papaya or pawpaw as it belongs to the plant family caricaceae. Papaya is a tree-like plant with a sing steam that is growing from 5 to 10cm tall, it has spirally set of leaves that are narrowed to the top of the stem. Papaya contains a wide-ranging spectrum of phytochemicals that includes the enzymes in the latex, the carotenoids in fruits and seeds, the alkaloids in leaves, the phenolic compounds in fruits, leaves and shoots, as well as it includes Glucosinolates in seeds and fruits. The fruits are big oval in shape and they are similar to melon by having a central seed cavity thus sometimes it is known as pepolike berries. Fruits weigh up to 9.072kg, and green until ripe, turning yellow or red-orange. Flesh is yellow-orange to salmon (pinkish-orange) at maturity. The edible portion surrounds the large, central seed cavity. The whole papaya plant contains a wide variety of pharmacologically active constituents. It contains a high nutritional value that helps to prevent the oxidation of cholesterol. “This plant is cultivated almost in all tropical and subtropical countries of the world particularly in India, Philippines, Srilanka, Nigeria, and Tanzania etc.” (Subenthiran et al. 2013). Papaya is used medically as a treatment of smooth upper respiratory tract ailment and so many diseases or infections such as psychiatric related illnesses, scorpion bites, hypertension toothache, tuberculosis, liver inflammations, arthritis and rheumatism. Crushed Carica papaya leaves have been used for anthelmintic purpose and fever. The main active consistent in papaya leaves is the macrocyclic lactone carpaine. This compound can reduce blood pressure and heart rate, movement of the intestinal strips and can also cause the uterus marked relaxation and the bronchiotes dilation.

“ Quercetin flavonoid is a plant pigment that is found in many plants and foods, such as red wine, onions, green tea, apples, berries, Ginkgo biloba and it is used as a medicine’’ (worts 2018). Quercetin flavonoids are phenolic substances, thus that definite types of cancer, metabolic disorder, cardiovascular diseases risks are reduced by the ingestion of flavonoids. “The Quecertin flavonoid is known for its anti-inflammatory, vasodilator and antihypertensive because of being the important bioflavonoids that is present in more than many plant materials” (Parasuraman et al.2016:89).

Thin layer chromatography

Thin-layer chromatography (TLC) is a highly used technique in synthetic chemistry for identifying compounds, determining their purity and following the progress of a reaction. It also permits the optimization of the solvent system for a given separation problem. It only requires small quantity of the compound and is much faster as well. “A thin layer chromatography uses a thin, uniform layer of silica gel or alumina coated onto a piece of glass, metal or rigid plastic” (Clark. 2007). The mobile phase is a suitable liquid solvent or mixture of solvents. Solvent are used for separation of mixture of strongly polar and nonpolar compounds. Methanol and acetone are one of the solvents that can be used in Thin-Layer Chromatography. Methanol is commonly used for extraction of bioactive and it is used for extraction of various polar compounds but certain group of nonpolar compounds are impartially soluble in methanol, if not freely soluble. This techniques is to check purity of given samples, it identifies compounds like acids, alcohols, proteins, alkaloids, amines, antibiotics, and more. It evaluates the reaction process by assessments of intermediates, reaction course, and so forth.

Advantages of TLC method

- It is a simple process that has a short development time.
- It helps with the visualization of separated compound spots easily.
- The method helps to identify the individual compounds.
- It helps in isolating most of the compounds.
- The separation method is faster and the selectivity of compounds is higher (even small differences in chemistry is enough for clear separation).
- The purity standards for the given sample can be assessed easily.
- It is a cheaper chromatographic method.

  System components of TLC

  - TLC plates, preferably ready made with stationary phase: these are stable and chemically inert plates, where a thin layer of stationary phase is applied on its whole surface layer. The stationary phase on the plates is of uniform thickness and is in a fine particles size.
  - TLC chamber, is used for the development of TLC plate. The chamber maintains a uniform environment inside for proper development of spots. It also prevent the evaporation of solvents and keeps the process dust free.
  - Mobile phase comprises of a solvent or solvent mixture. The mobile phase that is used should be particulate-free and of the highest purity for proper development of TLC spots. The solvent recommended are chemically inert with the sample, a stationary phase.

Retardant factor

A retardant factor is a value that is characteristic for any given compound (provided that it has the same stationary and mobile phases that are used). “This value provides corroborate evidence as to the identity of a compound” (Clack.2007). It is always known that the range of the value should be 0 to 1, the formula that is used to calculate the retardant factor is as follows:

Overview

Traditional plants contain various secondary metabolites such as phenolic, steroids, alkaloids and aterpenoids compounds. These compounds show antioxidant activities that include scavenging free radical species and they inhibit the production of reactive species that results from the normal cell metabolism. The present study was undertaken to analyse the thin layer chromatography and antioxidant activities of methanol extract of a leave of Carica papaya. The antioxidant activities were carried out by DPPH free radical scavenging assay, that includes OH* radical scavenging assay, NO* radical scavenging assay, Fe3+* reducing power assay and phosphomolybdeum reduction assay.

The tree of Carica papaya is always with soft main trunk and tufted leaves at the top. The papaya fruits vary in size, shape, colour and taste.

Antioxidants play an important role in a body defence system against reactive oxygen species (ROS) as they combine with reactive oxygen species and null their toxic effect. The reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide and other exogenous factors are generally the cause of several fatal disease such as coronary heart disease, stroke, rheumatoid arthritis, diabetes and cancer. Thus, any plant possessing antioxidant activity could be a potential lead for caring any of the above ailments.

Material, method and discussion

Leaves of Carica papaya were collected from porur, Chennai in India. The plant was authentised by prof.Dr.N. Raaman centre for advanced studies in Botany, University of madras, and Chennai in India. Leaves were thoroughly washed and dried in shade for 10 days. Dried leaves were made into coarse powder using mechanical blander and stored in air tight container till further use. The leaves were ground and powdered as a solvent by maceration method. Initially, coarse in methanol for 3 days. Then the supernatant was filtered through filter paper. Powdered extracts were concentrated using rotary evaporator and greenish-black coloured sticky residue was obtained.

Thin layer chromatography was carried out for methanol extract of leaves of c.papaya on Merck TLC aluminium sheets, silica gel 60 F254 (20x20 cm), precoated plates. The methanol extract of leaves of c.papaya was spotted at 0.3mm above from the bottom of the TLC plate. The chromatogram was developed in a mixture of suitable solvent system. The spots were visualized with ultraviolet light at 254nm. The Rf values of coloured spots were recorded.

The methanol extract of leaves of c.papaya was subjected to preliminary phytochemical screening using standard methods. The methanol extract of leaves of c.papaya was screened for different classes of phytoconstituents such as flavonoids, phenolic compounds, alkaloids, and Glucosinolates etc.

The total flavonoid content was determined by aluminium chloride ($AlCl_3$) method using Quecertin as a standard. The plant extract (0.1 mL) was added to the 0.3mL distilled water followed by 5% $NaNO_3$ (0.03mL). After 5 minutes at 250C, $AlCl_3$ (0.03mL, 10%) was added. After another 5 minutes, the reaction mixture was treated with 0.2mL of 1mM NaOH. Finally, the reaction mixture was diluted to 1 mL with water and absorbance was measured at 510nm. The

result was expressed as Quecertin equivalent. The radical scavenging assays were measured and their results were obtained and calculated using,

Conclusion [WU5]

The methanol extract of leaves of C. papaya showed the presence of significant amount of phenols and flavonoids.

Method

7 mg of Quercetin flavonoid and 5mg of dried Carica papaya were both weighed using an analytical balance. Solutions of 5mg/ml Carica papaya and 7mg/ml of Quercetin flavonoid were prepared in a conical tube using distilled water.

With a pencil, a thin mark was made at the bottom of the plates to apply sample spots. Then, the sample solutions of Quercetin flavonoid and Carica papaya were applied on the spots that were marked on the line in equal distances. Mobile phase of 90%acetone and 90%methanol were transferred into the TLC chambers to a levelled few centimetres above the chamber bottom in 5mL and the TLC chamber was covered with a film and waited for 20 minutes.

The plates that were prepared with sample spotting were placed in the TLC chamber so that the side of the plate with the sample line faced the mobile phase. Then the chamber was closed with a film.

The plate was then immersed, such that the sample spots were well above the level of mobile phase (but not immersed in the solvent) for development. Sufficient time was allowed for the development of spots. Then the plates were removed from the chamber and allowed to dry. The sample spots were seen under an ultraviolet light. This was done to 70%, 80%, and also 95% of methanol.

Discussion

The solvent systems of 90% acetone and 90% methanol were used and compared in the TLC of aqueous extract of Carica papaya. In Plate 1, there was no resolution of the sample by 90% of acetone as it did not separate the compounds of the sample whereas in plate 2, the spots did not appear at all by the 90% methanol solvent. The concentration of the sample was increased in plate 2 and it was then detected using the ultraviolet light. In plate 1, the sample had the same affinity to the stationary phase when the 90% acetone solvent travelled to the stationary phase. In plate 2, the sample had a high affinity to the stationary phase as it travelled slowly by the 90% methanol solvent, thus that the methanol was found to give the best resolution for the extract of Carica papaya. Different compositions of methanol were used such as 70%, 80%, 90% and 95%. All of the solvents were used in the TLC plates using the same samples and they were detected under an ultraviolet light of 254-365nm, where it was found that 95% of methanol had a great resolution of Carica papaya extract than all of the other methanol solvents. On the plate that used 95% methanol, the distance travelled by the sample and solvent were measured. It is known that the retardant factor value will always be in the range 0 to 1, if the substance moves and it should only move in the direction of the solvent flow and cannot move faster than the solvent. Thus that in this experiment the substance travelled 3.8

cm and the solvent travelled 4.15 cm, the retardation factor would be 0.9.