ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

# Seclusion of bioactive complexes from *Annona muricata* leaf extract using TLC

**Sharadha Priya Vardhini Rajsekhar, Helan Chandra. J*and Sridhar.S**
Department of Biotechnology, Jeppiaar Engineering College, Chennai, TamilNadu, India
***Corresponding Author: Email ID: helananand@gmail.com**

**ABSTRACT**

The current study embraces the extract of *Annona muricata* leaf using different solvents like chloroform, ethanol and double distilled water. The product yield was calculated and thin layer chromatography (TLC) technique was used to separate the phytocompounds from the crude leaf extract. This technique is being followed from early ages of research and it is a pioneer in the preliminary step in identifying the constituents present in the mixture. The mobile phase used in TLC can be either a combination of two or more solvents depending on the sample extract. The permutation used here were various combination of organic solvent tried. Mobile phase used here were toluene: ethyl acetate, chloroform: acetone, chloroform: ethyl acetate, hexane: acetone, chloroform: methanol and many more. The best result was analysed in the combination of toluene: ethyl acetate in ratio of 9:1. Retention Factor ($R_F$) was calculated for each and every combinations of the single compound that travels.

**KEY WORDS**: *Annona muricata*, phytocompounds, TLC, mobile phase, $R_F$

## 1. INTRODUCTION

Medicines are any substances that are meant to change the way your body system deals with an illness or injury or to maintain your health and wellbeing, where the source of origin can be any natural (including plant kingdom, microbes, oceanic marine organism) synthetic, chemical combinations or by alternative medicinal system which includes Ayurveda, Siddha, Homeopathy, Naturopathy, Yoga, Unani, with minor side effects. Plant was abundant source for huge amount its phytoconstituents can be used in the arena of medicine. Many biochemical compounds were present in plant source. The basic principle behind the techniques in separation of the bioactive compounds from crude sample by miscellaneous methods includes solvent system, gravitational force, centrifugal force and other methods (Boyom, 1996). One of the oldest techniques used in separation of the phytocompounds from the crude extract was Thin Layer Chromatography (TLC) and till date (Harborne, 1998). The screening of all the bioactive compounds from flora kingdom in initial strategy is TLC.

*Annona* is a genus of the Annonaceae, of which there are about 129 species distributed mainly in Tropical and subtropical region. The fruit is very delicate dark green covered with soft spines. The flesh is white, creamy, meaty, juicy and slightly acidic, measuring 2-3 cm long, can weigh 2.5 kg (Ramesh et al 2013). All plant parts are used in natural medicine, including bark, leaves, roots and fruits, but the part that contains the greatest concentration of active ingredient is in the leaf, where the Annonaceae Acetogenins, which have been widely studied from 1940 that came into use as an insecticide, leading to surprise the research scientists for its broad power, without causing any harmful effects in animals and man (Gajalakshmi, 2012; Pélissier 1994).

Soursop, leaf and bark powder cure diabetes by regulating blood sugar, which shows its high effectiveness in endocrine commitments: liver, kidney, thyroid, pancreas, ovary, prostate, intestines, muscle relaxant smooth (heart), gall bladder, appendix and fights lung cancer or Lewis, breast cancer and brain tumours, hypotensive, antispasmodic, vasodilator, eliminates dust mites that cause asthma and bronchial diseases (Ezejindu, 2014). The stems, leaves and roots are considered sedative, hypotensive (blood pressure lowering), antispasmodic and ant diabetic. The leaves are used as a tea against catarrh (inflammation of mucous membranes). The ground seeds are used by Andean tribes against intestinal parasites (Minari and Okeke, 2014).

## 2. MATERIALS AND METHDOLOGY

**Sample collection and extract preparation:** *Annona muricata* leaves were collected from the district of Nagercoil, Tamilnadu. The crude extract was done with 100 gm. of shade dried fine grounded leaf powder using organic solvent extraction like chloroform, ethanol and double distilled water in Soxhlet apparatus. The extraction was purified by Whatmann filter paper No: 1 to remove the impurities which might be present during the process (Consolacion, 2014).

**TLC Plate specification:** The TLC analysis was carried out on TLC plate [Merck $F_{254}$ Silica gel 60 (size=20×20; thickness=≤30)]. The sample was taken in capillary tube and the sample spots were placed on plates (Neroth, 1963). The mobile phase was prepared with combination of solvents which were tabulated in Table-1. This plate was placed inside the beaker and the lid was closed in order to increase the efficiency of the lead bioactive metabolite to reach the saturation limit in the plate. After the sample run on the plate was stable, the solvent run on the plate was marked (Bipin D Lade, 2014). All the compounds separated from the crude were labelled on the plate to calculate the Retention Factor ($R_F$) values. The $R_F$ value of the bioactive compound is defined as the distance travelled by the compound divided by the distance travelled by the solvent (Mali, 2013). The plates were kept in the chamber with

Iodine crystals for few seconds, the mobile phase and the metabolites react with iodine and the compounds were visible under the UV light. The visibility of these various compounds was determined within few seconds (Harborne, 1998; Cai, 2014).

## 3. RESULTS AND DISCUSSION

The yields of the solvent extracts like ethanol, chloroform and double distilled water were calculated using dry and wet weight. The dry weight of the extracts from various solvents ethanol, chloroform and water were found to be 97g, 95.54 g and 95 g respectively. The yield of solvent extracts like ethanol, chloroform and aqueous were 3g, 4.5g and 5g respectively.

With many combinations of solvents that were tried and the list of all the combination with respective to ratio were entitled in the Table-1 (Consolacion, 2014). Out of all the combinations only two blends of the solvent gave the best results-Toluene: ethyl acetate (9:1) and chloroform: Acetone (9:1). While Petroleum Ether: Ethyl Acetate: Acetic Acid solvent combination system gave least results and other system of solvent mixture didn't result out appropriately. The following are the calculated $R_F$ value by thin layer chromatographic plates in mobile phase Toluene:Ethyl acetate (9:1) and chloroform: acetone (8:2) in Table-2 and Table-3 respectively.

*Annona muricata* crude leave extract made with chloroform, ethanol and aqueous were subjected simultaneously on the TLC plate with many different solvent combinations as mobile phase for the study. Out of 12 combinations of solvent mixture used here in mobile phase, only two solvent systems of mobile phases gave the best result in separating the bioactive compounds from the crude plant sample extract.

The two different solvent systems of mobile phase used here in this of Thin Layer Chromatography studies were Toluene: Ethyl acetate (9:1) and Chloroform: Acetone (9:1). The $R_f$ value calculated for the toluene: ethyl acetate (9:1) solvent combination of the Chloroform extract of the *Annona muricata* leaf extract found to be maximum value with 0.905±0.033 and minimum of 0.073±0.003, for the ethanolic extract the maxima was 0.902±0.023 and minima to be 0.122±0.04 and for the chloroform: acetone (9:1) mobile phase, the maxima calculated 0.845±0.029 and minima to be 0.087±0.012 in chloroform extract, in the ethanolic extract the higher value calculated to be 0.854±0.030 and the lower value calculated to be 0.465±0.033 and no band separation was identified in the aqueous extract in both the solvent systems of crude extract. The standard used here were rutin and quercetin, which were flavonoids. The $R_F$ value for rutin was 0.13±0.05 and quercetin was 0.93±0.04 (Touchstone and Levine, 2006). The values found for the plant extract for both ethanol and chloroform was verified to that of the standard. Acetogenins, which was the active ingredient present in the abundant quantity in *Annona muricata*, used in treating in 7 different types of cancer till date and its $R_f$ value was 0.79±0.05 (Melot A, 2009).

**Table.1.The list of Solvent Combinations that were used TLC studies**

| Solvent combination | Ratios in V:V |
|---|---|
| Chloroform : Acetone | 9:1 |
| Toluene : Ethyl Acetate | 9.3:0.7 |
| Chloroform : Ethyl Acetate | 8:2 |
| Toluene : Ethyl Acetate | 9:1 |
| Hexane : Acetone | 9:1 |
| Chloroform: Acetone: | 8:2 |
| Hexane: Ethyl Acetate | 9:1 |
| Chloroform : Methanol | 8:2 |
| Chloroform: Hexane | 8:2 |
| Ethyl Acetate : Hexane | 7:3 |
| Petroleum Ether: Ethyl Acetate: Acetic acid | 8:2:2 |
| Dichloromethane : Methanol | 7:3 |

**Table.2.The calculated $R_f$ Value were for the solvent combination toluene: ethyl acetate (9:1)**

| Solvent Combination | Solvent Extract | $R_f$ Value |
|---|---|---|
| Toluene : Ethyl Acetate ( 9:1) | Chloroform | 0.905±0.033 |
| | | 0.863±0.044 |
| | | 0.663±0.0387 |
| | | 0.648±0.029 |
| | | 0.530±0.035 |
| | | 0.116±0.025 |
| | | 0.073±0.003 |
| | Ethanol | 0.902±0.023 |

| | | |
|---|---|---|
| | | 0.814±0.028 |
| | | 0.732±0.036 |
| | | 0.713±0.014 |
| | | 0.549±0.038 |
| | | 0.122±0.04 |
| | Double Distilled Water | - |

**Table.3.The $R_f$ Value were calculated for the solvent combination Chloroform: Acetone (9:1)**

| Solvent Combination | Solvent Extract | $R_f$ Value |
|---|---|---|
| Chloroform : Acetone (9:1) | Chloroform | 0.845±0.029 |
| | | 0.814±0.017 |
| | | 0.721±0.026 |
| | | 0.239±0.035 |
| | | 0.146±0.020 |
| | | 0.087±0.012 |
| | Ethanol | 0.465±0.033 |
| | | 0.835±0.025 |
| | | 0.854±0.030 |
| | Aqueous | - |

## 4. CONCLUSION

In the present investigation, the report of the TLC was analysed that the seven bands were observed with the chloroform elute in toluene:ethyl acetate in V:V (9:1). The bioactive compound that is been separated was compared to that of standard compound like rutin (0.13) and quercetin (0.93), $R_F$ values were compared and that the compound isolated wasn't the standard. These bioactive compounds can be isolated using TLC, which is a efficiency, commercial and cost effective method to seclude the compounds.

## REFERENCES

Alice Melot, Djibril Fall, Christophe Gleye and Pierre Champy, Apolar Annonaceous Acetogenins from the Fruit Pulp of Annona muricata, 14(1), 2009, 4387-4395.

Bipin D Lade, Anita S Patil, Hariprasad M Paikrao, Ankit S Kale, Kushal K Hire "A Comprehensive Working, Principles and Applications of Thin Layer Chromatography, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5(4), 2014, 486.

Boyom FF, Zollo PHA, Menut C, Lamaty G, Bessière, JM, Aromatic plants of tropical central Africa. Part XXVII. Comparative study of volatile constituents of five Annonaceae species growing in Cameroon, Flavour and Fragrance Journal, 1996.

Cai L, Thin Layer Chromatography. Current Protocols Essential Laboratory Techniques, 2014, 6.3.1–6.3.18

Consolacion Y. Ragasa, Richard F, Galian and Chien-Chang Shen, Chemical constituents of *Annona muricata*, Der PharmaChemica, 6(6), 2014, 382-387.

Ezejindu DN, Udemezue OO, Chukwujekwu IE, Uchefuna RC, Maduka SO, Akingboye AJ, The histological effects of *Annona Muricata* (Soursop) on the Adrenal Gland of Adult Wistar Rats, International Journal of Biomedical And Advance Research, 5(10), 2014, 1150-1155.

Gajalakshmi S, Vijayalakshmi S and Devi Rajeswari V, Phytochemical and pharmacological properties of annona muricata: a review, International Journal of Pharmacy and Pharmaceutical Sciences, 4(2), 2012, 556-559.

Harborne JB, Photochemical methods: a guide to modern techniques of plant analysis. Edn3, Chapman and Hall, London, 1998, 182-190.

Mali. B. D. Specific Spray Reagent for the Detection and Identification of Endosulfan by Thin-Layer Chromatography, Journal of Planar Chromatography, 26(6), 2013, 508–509.

Minari JB, Okeke U, Chemopreventive effect of Annona muricata on DMBA-induced cell proliferation in the breast tissues of female albino mice, The Egyptian Journal of Medical Human Genetics 2014.15, 327–334.

Neroth P, Thin-layer chromatography of bile acids. Journal of Lipid research, 4(1), 1963, 11-16.

Pélissier Y, Marion C, Kone D, Lamaty G, Menut C, Bessière JM, Volatile components of *Annona muricata* L. Journal of essential oil research, 6, 1994, 411-414.

Touchstone JC and Levin SS, Sample Application in Thin Layer Chromatography. Journal of Liquid Chromatography, 3(12), 2006, 1853-1863.

Vijayameena C, Subhashini G, Loganayagi M and Ramesh B, Phytochemical screening and assessment of antibacterial activity for the bioactive compounds in *Annona muricata*, 2, 2013, 1-8.