Bangladesh J Pharmacol. 2016; 11: 844-848

VISUAL EXPERIMENT

Isolation and purification of plant secondary metabolites using column-chromatographic technique

Vivek K. Bajpai[1], Rajib Majumder[1] and Jae Gyu Park[2]

[1]Department of Applied Microbiology and Biotechnology, Yeungnam University, Gyeongsan, Gyeongbuk 712-749, South Korea; [2]Pohang Technopark, Pohang Center for Evaluation of Biomaterials, 394, Jigok-ro, Nam-gu, Pohang, Gyeongbuk, South Korea.

Correspondence to Vivek K. Bajpai at vbiotech04@gmail.com

First Published: 1 October, 2016

DOI: 10.3329/bjp.v11i4.28185

ABSTRACT

Chromatographic techniques have significant role in natural products chemistry as well as contribute dramatically in the discovery of novel and innovative compounds of pharmaceutical and biomedical importance. This study focused on step-by-step visual demonstration of fractionation and isolation of biologically active plant secondary metabolites using column-chromatographic techniques. Isolation of bioactive compounds using column-chromatographic involves: a) Preparation of sample; b) Packing of column; c) Pouring of sample into the column; d) Elution of fractions; and e) Analysis of each fractions using thin layer chromatography. However, depending on nature of research, compounds can be further purified using high performance liquid chromatography (HPLC), and nuclear magnetic resonance (NMR) spectral analyses.

INTRODUCTION

Plants are healthy and natural resource of life (Russell and Duthie, 2011). In particular, medicinal plants are of great importance with endless therapeutic properties useful for curing various diseases with an advantage of being natural (El-Shemy et al, 2007). At present, there are un-countable products in market, with adverse side effects on once heath. Therefore, the use of secondary metabolites from plant origin could be an advantage and best solution to narrow down the use of unhealthy products (Russell and Duthie, 2011).

In past, the plant or microbial extracts in crude or partially-purified forms were the only sources of medication available for the treatment of human and animal diseases. This gave an idea that the effect of a drug in human body is due to an interaction of drug with biological molecules. This opened new doors in pharmacology, as pure, isolated chemicals, instead of extracts, as the standard for the treatment of diseases. At present, there are innumerable number of such bioactive compounds isolated form crude extracts and their chemical structure were elucidated (Bajpai et al., 2001).

Moreover, plants have always been a source of a wide array of secondary metabolites with potential pharmacological properties (Russell and Duthie, 2011). Polyphenolic (flavonoids) compounds occur ubiquitously in foods of plant origin have many beneficial health effects due to their potential anti-oxidant, anti-inflammatory and cancer-preventive activities (Li et al., 2014).

Therefore, the objective of this study was to provide step-by-step visual demonstration of fractionation and isolation of biologically active plant secondary metabolites using column-chromatographic techniques.

MATERIALS AND EQUIPMENTS

1. Cylindrical chromatographic column
2. Separating funnel

3. Silica gel 60 (Mesh 230-400)
4. *n*-Hexane (need for packing of column)
5. Cotton or silica sand
6. *n*-Hexane (need for packing of column)
7. Iodine chamber
8. UV light detector (254 nm)
9. TLC plate developing chamber
10. Lead pencil
11. Capillary tubes
12. Spatula
13. Organic solvents
14. Preparative TLC plates (20 cm x 20 cm)
15. Kim wipe tissue
16. Solvent mixer

PREPARATION OF REAGENTS

Test sample: Air dried sample powder was extracted using suitable solvent (depending on the nature of the work) and vacuum dried before loading into column. Amount of the sample should be measured in order to define the yield of desired extract.

Gradient solvent system: Gradient solvent system (non-polar to high polar solvent system) provides best elution and best separation of various organic compounds from any plant-based organic extract. However, volume of solvent can be dependent on the amount of the sample to be purified. Table I summarizes the ratio of gradient solvent to be used in column chromatography.

Table I

Gradient solvent system to be used in the column-chromatography for the isolation of bioactive molecules from any test samples

Solvent system	Ratio	Volume (mL)	Fraction
Hexane	100%	50	1
Hexane: Ethyl acetate	10:1	50	2
Hexane: Ethyl acetate	5:1	48	3
Hexane: Ethyl acetate	1:1	50	4
Hexane: Ethyl acetate	1:5	48	5
Hexane: Ethyl acetate	1:10	50	6
Ethyl acetate	100%	50	7
Ethyl acetate: Methanol	10:1	50	8
Ethyl acetate: Methanol	5:1	48	9
Ethyl acetate: Methanol	1:1	50	10
Ethyl acetate: Methanol	1:5	48	11
Ethyl acetate: Methanol	1:10	50	12
Methanol	100	50	13

Volume of the solvent system is dependent on the amount of the sample to be purified

Organic solvents: Ready to use and purchased commercially from Sigma (MO, USA)

VIDEO CLIPS

Part 1: 3 min 45 sec

Part 2: 6 min 21 sec

Part 3: 4 min 45 sec

METHOD

Column-chromatography

A cylinder shaped glass column containing stationary phase (silica gel) is encountered slowly from the top with a liquid solvent (mobile phase) that flows down the column with the help gravity or external pressure applied. This technique is used for the purification of compounds from a mixture. Once the column is ready, the sample is loaded inside the top of the column. The mobile solvent is then allowed to flow down through the column. The compounds in mixture have different interactions ability with stationary phase (silica gel), and mobile phase, thereby will flow along the mobile phase at different time intervals or degrees. In this way, the separation of compounds from the mixture is achieved. The individual compounds are collected as fractions and analyzed further for structure elucidation.

PROTOCOL

Isolation and purification of bioactive compounds from plant samples

1. A suitable size long cylindrical glass column (based on the amount of the sample) should be stand firm on a column-chromatography stand.
2. Completely dried plant extract sample should be mixed with silica gel to make a fine powdered form for easy distribution of sample in already packed silica gel column.
3. Sample powdered mass should be placed on the top of the pre-packed silica column and sample should be covered with a layer of cotton.
4. Then solvents of different polarities were passed through column at uniform rate under gravity to fractionate the sample extract.
5. Each fraction was collected separately in a test tube and numbered consecutively for further analysis on thin layer chromatography.
6. Thin layer chromatography (TLC) provides partial separation of both organic and inorganic materials using thin-layered chromatographic plates especially useful for checking the purity of fractions.
7. Each fraction is applied on activated TLC plates with the help of capillary tube at a 1/2 inch apart from the lower edge of TLC plate, and plate is kept in a developing chamber containing suitable solvent system for specific time until the developing solvent reaches top of the upper edge of TLC plate.
8. Plate is taken out from developing chamber, dried and solvent front is marked by lead pencil. Compound bands/spots visualized on TLC chromatoplate can be detected by visual detection, under UV light (254 nm), in iodine chamber and by using spray reagent (vanillin-sulfuric acid) for the presence of specific compounds.
9. The visualized spots of the components in the chromatoplate are marked and the R_f value of each spot is calculated by the formula: R_f = distance travelled by the sample (cm)/distance travelled by the solvent (cm).
10. TLC plate showing number of bands (compounds) for each fraction can be further purified using high performance liquid chromatography (HPLC).
11. Based on the nature of the compounds, further spectral analyses such as infrared (IR), mass spectrometry, and nuclear magnetic resonance (NMR) can be performed to elucidate the chemical structure of target compounds.

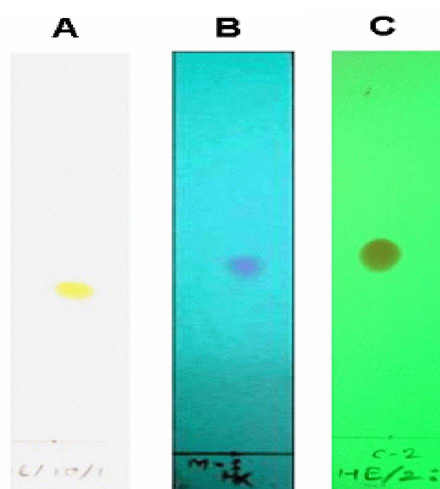

Figure 1: Demonstration of isolation pattern of pure compounds on TLC plate using iodine (A), UV-detection (B) and spray reagent (C)

DISCUSSION

Not all secondary metabolites or natural products such as penicillin, morphine, and paclitaxel (Taxol) can be fully synthesized due to their very complex structures that are too difficult and expensive on industrial scale. Hence, there is an urgent need to search for alternative remedies as naturally occurring biologically active secondary metabolites from plant origin. Certain phytochemicals act in many ways on various types of disease complex, and may potentially contribute in the field of pharmacology as natural supplements to control various infectious diseases in future as the fast and reliable alternatives. Many non-natural, and synthetic drugs cause severe side effects that were not acceptable except as treatments of last resort for terminal diseases such as cancer. The metabolites discovered in medicinal plants may avoid the side effect of synthetic drugs, because they must accumulate within living cells (El-Shemy et al., 2007). Considering the deleterious effects of synthetic antibiotics, isolation, purification and characterization of novel types of plant secondary metabolites could be a safer alternative to synthetic compounds (Bajpai and Kang, 2011). Various chromatographic techniques have been used for successful fractionation and purification of biologically active compounds from variety of sample. Column-chromatography is one of the most popular and widely used separation techniques to characterize both organic and inorganic materials suggesting is potential usefulness in chemical analysis of complex extract material. This research visualized successful application of column-chromatographic techniques for the isolation of biologically active secondary metabolites from plant sample. However, ongoing investigation of toxic or irritant properties is imperative, especially when considering any new biologically active compound for human use, by them medicinal or otherwise.

REFERENCES

Bajpai VK, Kang SC. Isolation and characterization of biologically active secondary metabolites from *Metasequoia glyptostroboides* Miki ex Hu. J Food Safety. 2011; 31: 276-83.

El-Shemy HA, Aboul-Enein AM, Aboul-Enein KM, Fujita K. Willow leaves' extracts contain antitumor agents effective against three cell types. PLoS ONE 2007; 2: e178.

Li AN, Li S, Zhang YJ, Xu XR, Chen YM, Li HB. Resources and biological activities of natural polyphenols. Nutrients 2014; 6: 6020-47.

Russell W, Duthie G. Plant secondary metabolites and gut health: The case for phenolic acids. Proc Nutr Soc. 2011; 70: 389-96.

PRECAUTION

Precautions should be taken while pouring the slurry of silica gel in to the column to ensure no formation of air bubbles.

Silica gel 60 (mesh 230-400) should be activated in an electric oven at 110°C for an hour before making the slurry to pack the column.

The surface of the column should not be allowed to dry in order to achieve a proper elution of fractions.

A constant flow rate of elution should be maintained throughout the column chromatographic process.

Elution should not be stopped during column chromatographic process this may cause cracks and air bubble formation thus may have negative impact on separation.

While spotting pure fraction of samples on TLC plates, capillary tube should be washed 2-3 times after each spotting to avoid cross contamination.

Make sure that the sample was not washed away when the TLC plates were placed in developing tank.