DETERMINATION OF SDG BY THIN LAYER CHROMATOGRAPHY EXTRACTED FROM *Linum usitatissimum.*

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ABSTRACT *Flaxseed (Linum usitatissimum L.) is a multi-purpose crop and its consumption is beneficial for human health. The nutritional components of flaxseed are oil, protein, lignans, fiber and vitamin. The determination of the minor components is of great importance in establishing the flaxseed oil quality and their genuineness.*

SDG – Secoisolariciresinol diglucoside is the principle lignan present in flax seeds. The Identification methods vary from simple to complex. To isolate SDG, flax seeds were defatted by four volumes of n-hexane with continuous stirring and the wet cake was collected. Then the wet cake was heated at 70°C and added 80% (v/v) aqueous methanol, brought to alkaline pH by adding sodium hydroxide with continuous stirring for about 3 hrs to extract maximum yield of SDG. Neutralization was done by adding a few drops of conc. H_2SO_4 (pH 5-6), distilled under vacuum in a rotary evaporator. The isolated lignan was purified by column chromatography using ethanol, ethyl acetate as elution system. Desired lignan fractions were collected combined and the solvent was evaporated in a lyophilizer to get the colorless solid portion which was identified by TLC. This study was conducted to enhance a suitable TLC method to determine SDG extracted from Linum usitatissimum.

Keywords: *Flax seed; Lignan; secoisolariciresinol diglucoside and TLC.*

1. INTRODUCTION

Flaxseed is the seed from the flax plant (*Linum usitatissimum L.*), which is a member of the Linaceae family. The plant is not a new crop and native to West Asia and the Mediterranean Berglund, 2002 [1]. Flaxseed is rich in fat, protein and dietary fiber. Chemical analysis of flaxseed averaged 30 to 40% oil, 20 to 25% protein, 20 to 28% total dietary fiber, 4 to 8% moisture and 3 to 4% ash and the oil contains vitamins A, B, D and E, minerals and amino acids. By virtue of the presence of physiologically active food components that may provide health benefits beyond basic nutrition, flaxseed is often grouped into one of several categories: ''functional food'', ''bioactive food'' and an ''endocrine active food'' [2] Hasler et al., 2000.

Lignans are a group of poly phenolic compounds in plants that share structural similarities with estrogen and thus have been classified as phyto estrogens. There are two main types of lignans found in flaxseed, secoisolariciresinol diglycoside and matairesinol, which are contained primarily in the seed coat [4] Sicilia et al., 2003. The level of SDG in flaxseed, 1–4% (w/w) Eliasson et al., 2003 [3], is 60–700 times higher than that in other edible plant parts. Variation in flaxseed lignan concentrations depend on the variety, location, and crop year [5]Westcott and Muir, 1996b. Secoiso lariciresinol (SECO) amount found in foods. Whole seed and ground flax typically contain between 0.7% and 1.9% SDG. Lignan are part of large structures such as dimers, trimers, or higher oligomers. The lignans from flaxseeds are linked within an oligomeric structure called the lignan macromolecule [6] Struijs et al. 2007, in which it is covalently bound via ester linkages to 3- hydroxy-3-methyl glutaryl (HMG), A straight chain oligomeric structure composed of 5 SDG residues interconnected by 4 HMGA residues (molecular weight of about 4000 Da) was also reported [7] Kamal- Eldinet al. 2001. SDG is the major lignan found in flax seed, which is known to have antioxidant [8, 9], anti-hyperglycemic [10, 11] and anticancer properties [12].

The aim of this study is isolation, extraction and Identification of SDG from flax seed by TLC and the purification studies were carried by column chromatography method. Thin layer chromatography is extensively used as simple chromatographic technique for qualitative analysis. TLC has many advantages in analyses including ease of operation, detection and confirmation without interfering the mobile phase and cost effectiveness. Many samples can be analyzed on a single plate with low solvent usage.

2. MATERIALS AND METHODS

2.1 Materials:

Flax seeds were collected from local market. The solvents and other chemicals used are n- hexane, methanol, ethyl acetate, ethanol, sulfuric acid, sodium hydroxide and silica gel which were obtained from Merck. SDG standard used was from Chengdu Bio purify Phytochemicals Ltd. Wenjiang Zone, Sichuan, China

2.2 Methodology:

Preparation of SDG extract:

a) Cleaning of flax from derbies which include other plants seeds, some parts of vegetarian of flaxseed and dust, Secondly grinding flaxseeds properly by a grinder machine eventually obtained on a homogenized powder that was ready for extraction. This stage involved de-fatting of flax oils by adding n-Hexane with continuous stirring for 30 min, filtered through a Buchner funnel by using Whatman filter paper No.1, the filtrate was discarded and the wet cake collected by using Soxhelt apparatus. Extraction of crude Lignan by the method which was described by [13] Rickard et al, (1996), involves taking 25 g of defatted powder treated with 80% (v/v) aqueous methanol, sample put on magnetic stirrer at 70°C for 3 hrs., then neutralized the reaction mixture by adding 12 g of Sodium Hydroxide (pH ≥ pH10)to obtained crud lignan.

b) Separation of Lignan: The process of separation by alkaline hydrolysis of SDG oligomers according to (Li et al. ,2008 and Yuan et al..,2008) by using an alkaline hydrolysis solution (a methanolic NaOH , 30 mM, pH=10) at 70 ºC for hydrolyzing SDG oligomers. The mixture was filtered by whatman filter paper no.1 then the supernatant was concentrated with a rotary evaporator within 45^0 C. Eventually, a thick sticky texture material , pH was adjusted to 5.0 by adding drops of conc. sulfuric acid then the sample was stored in 4^0 C. Evaluation was done by TLC.

3. RESULTS AND DISCUSSION

3.1 Thin-layer chromatography

Thin-layer chromatography (TLC) has been used in lignin research since the 1960s. It is a simple and inexpensive technique and is particularly suitable for screening of a large number of samples, and for monitoring isolation procedures. TLC is routinely used for a first qualitative examination of plant extracts. Quantitative determination can be achieved by densitometric detection. The most used phase has been silica gel. A wide range of eluents and detection techniques have been applied. Preparative TLC is much practiced for the isolation and purification of small amounts of lignans and other polyphenols. Lignans all absorb UV light and can thus be detected using 254 nm UV light. Spraying with a 5% solution of sulphuric acid in ethanol is also commonly used.

In our studies of lignans, we have found TLC valuable for fast screening of extracts and for preparative separation of lignans for further studies by GC and HPLC. For example, we used TLC on RP-plates (RP-8) for preparative isolation of lignans occurring in small amounts in extracts. TLC provides a good overview of the lignan pattern, although all lignans cannot be separated. The separation is governed mainly by the number of hydroxylgroups, but lignans with the same number of hydroxyl groups may also be separated. Identification of the predominant lignans is facilitated by the specific colors obtained by spraying with sulphuric acid in ethanol followed by rapid heating in an oven.

3.2 Evaluation by TLC:

TLC is usually performed in one-dimension by gravity-flow ascending development with a single mobile phase in a solvent vapor-saturated, paper-lined glass N-chamber Multiple development in one direction with the one or more solvents can improve resolution and shape of the spots some times.

A portion of the resulting residue was dissolved it in ethanol and compared with standard by TLC-Thin layer chromatography method [8] by using solvent/mobile phase as Ethanol: Ethyl acetate (90:10) ratio. The resulting TLC pattern was in band manner as shown in Figure 1.

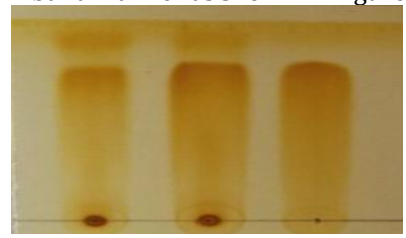
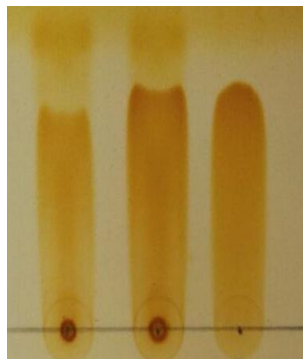


Figure 1: 1.Sample 2.Co Spot 3.Standard

TLC was performed again by using solvent/mobile phase as Ethanol: Ethyl acetate (99.5:0.5) ratio to get the spots as desired. The resulting TLC was again observed in band manner for fraction/sample, Co-spot and Standard as shown in Figure 2.



↓ ↓ ↓
1 2 3

Figure 2: 1.Sample 2.Co Spot 3.Standard

Further TLC was performed by introducing aqueous medium to the mobile phase [9] by using solvent/mobile phase as Ethyl acetate: Methanol: Ethanol: Aqueous (81: 11: 4: 8) ratio to get the desired spots. The resulting TLC was not in band manner and spots were observed for fraction/sample, Co-spot and Standard as shown in Figure 3.

↓ ↓ ↓
1 2 3

Figure 3: 1.Sample 2.Co Spot 3.Standard

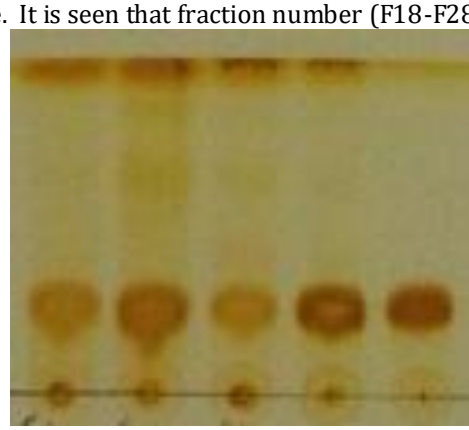
The extract was tested by using TLC. The plant extracts were spotted using a capillary tube on TLC plates. The plates developed by Ethanol: Ethyl acetate (90: 10), (99.5: 0.5) and Ethyl acetate: Methanol: Ethanol: Aqueous (81: 11: 4: 8) ratios were then viewed under UV fluorescence light at wavelength 254 nm and also exposed to iodine vapors in a chamber; Rf= 0.24.The resulting fraction can be purified further by obtaining various purification techniques.

3.3 Purification by Column chromatography technique:

A glass column with diameter of 20 mm length x 90 cms was clamped upright and packed with silica gel of GF 254 of Merck mixed with the appropriate mobile phase and poured into the column as a compact uniform suspension. This constituted the stationary phase. The extract was then mixed with a small amount of the mobile phase and loaded as a thin band to the silica gel. Once the extract was introduced onto the silica gel, the mobile phase was added at a constant flow rate. Gradient elution of increasing polarity was initiated consisting of successive elution of several fractions using different solvent mixtures composed of n-hexane, ethyl acetate and ethanol.

The ethanol fraction was subjected to column chromatography on silica gel and eluted with stepwise gradient polarity using n-Hexane: Ethyl acetate in ratios of 100:0, 80:20, 60:40, 30:70; 20:80; and

0:100. 50 ml fractions were collected for the different ratios mentioned as solvent system to the given 16 fractions (F1-F16). The system was further eluted with Ethyl acetate: Ethanol in ratios of 100:0, 95:5, 90:10, 80:20, 70:30, 50:50, 30:70; 20:80; and 0:100. 50 ml fractions collected for the different ratios mentioned to give 32 fractions (F17-F48). These fractions were then further subjected for TLC studies (Figure 4) and compared with authentic sample. It is seen that fraction number (F18-F28) contained the major compound.



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1 2 3 4 5

Figure 4 1,2,3. Sample Fractions 4.Co Spot 5.Standard

These fractions were combined together and the solvent was evaporated to get the colorless solid by using SP Scientific lyophilizer and 94.3% of compound purity was achieved by HPLC [14-18].

4.0 CONCLUSIONS

TLC is a simple, inexpensive and rapid method is applied mostly for a qualitative examination of seed extracts and for monitoring various stages of lignin purification. Qualitative TLC has been applied to elaborate lignin patterns in flax seeds.

To determine SDG in flax seeds, methanol solutions of extracts from seed (subjected to alkaline hydrolysis and acidified) were applied along with SDG slandered solution on silica gel plates and developed with Ethanol: Ethyl acetate (90: 10), (99.5: 0.5) and Ethyl acetate: Methanol: Ethanol: Aqueous (81: 11: 4: 8 v/v). The optimized method for the extraction in this study is not taking as much of time in accordance with many of the existing methods. Identification was done by TLC and the purification was done by column chromatography method which is a very economical and purity of the compound is found to be 94.3%, which is determined by HPLC.

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