

## **Extraction and Purification of lignan compound from flax seed *Linum usitatissimum***

**Essam F. Al-Jumaily<sup>a</sup> Ali O. A. Al-Shimary<sup>a</sup> and Esmail K. Shubbr<sup>b</sup>**

<sup>a</sup>Genetic Engineering and Biotechnology Institute for postgraduate studies –Baghdad University ,Iraq

<sup>b</sup>Ministry of Science and Technology, Faculty of Hazared Materials and Environment, Baghdad, Iraq

### **ABSTRACT**

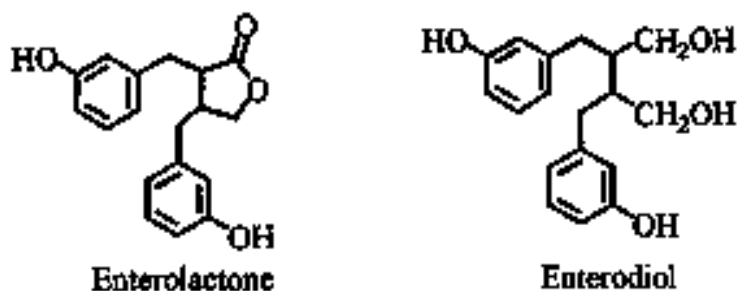
The extraction and purification of lignan (Secoisolariciresinol diglucoside, SDG) from flax seed (*Linum usitatissimum*) was made by using following chromatographic techniques: Liquid-liquid chromatographic, ion-exchange chromatographic, thin layer chromatographic (TLC) and high performance liquid chromatographic (HPLC).

**Keywords :** Lignan , Secoisolariciresinol diglucoside, flax seed, Chromatographic techniques.

### **INTRODUCTION**

Flax seed (*Linum usitatissimum*) is belongs to linaceae family. Flax seed is presently grown for its oil in additions, flax is a rich source of fatty acid and has increasing uses in foods. Flax seed suffers from the fact that the level of fatty acid instauration in the triglyceride oil is high and is subject to oxidative polymerization.

The industrial properties of linseed oil are legendary for use in linoleum and paint products [1]. There has been considerable interest shown in a class of minor compounds contained in flax seed collectively referred it as lignans. The first called lignan is Hawarth in 1936 which are dimmers formed the same monomeric species (phenylpropane) which coupling between the two carbon atoms of cinnamyl side-chain.[2]. By the oxidative reaction which products the bond [3]. Flax seed is one of the best dietary sources of lignans. The main lignan in flax seed is secoisolariciresinol diglucoside (SDG), which is present in large quantities[4]. The lignans are generally cinnamic acid dimmers containing a dibenzylbutane skeleton. When part of the human diet, contain lignans are believed to be converted into mammalian lignans known as enterolactone and enterodiol.[5]. (Fig.1).



**Figure.1: Molecular structural of lignans.**

The present study aimed to extracted and purify the compound of lignan from flax seed (*Linum usitatissimum*).

## MATERIALS AND METHODS

Flax seeds were obtained from the Al-Rabea'a central for Agriculture and Food Research .Industry Ministry. Which belong to (*Linum usitatissium* L.).The molish's ; Benedict's and fehlinf's test were prepared according to Rickard *et al*[6]. The hydrolyzing agent solution was done according to Rickard *et al.* [6].

A 25 gm sample of meal was extracted at 4 ° C by intermittent slurring with a mixture of Diexam:ethyl alcohol (1:1) ratio of (1:8) for 24 hours. After that the suspension was filtered and the extract was evaporated under reduced (40 °C) pressure to produce a dry crude lignan (Scheme 1.)

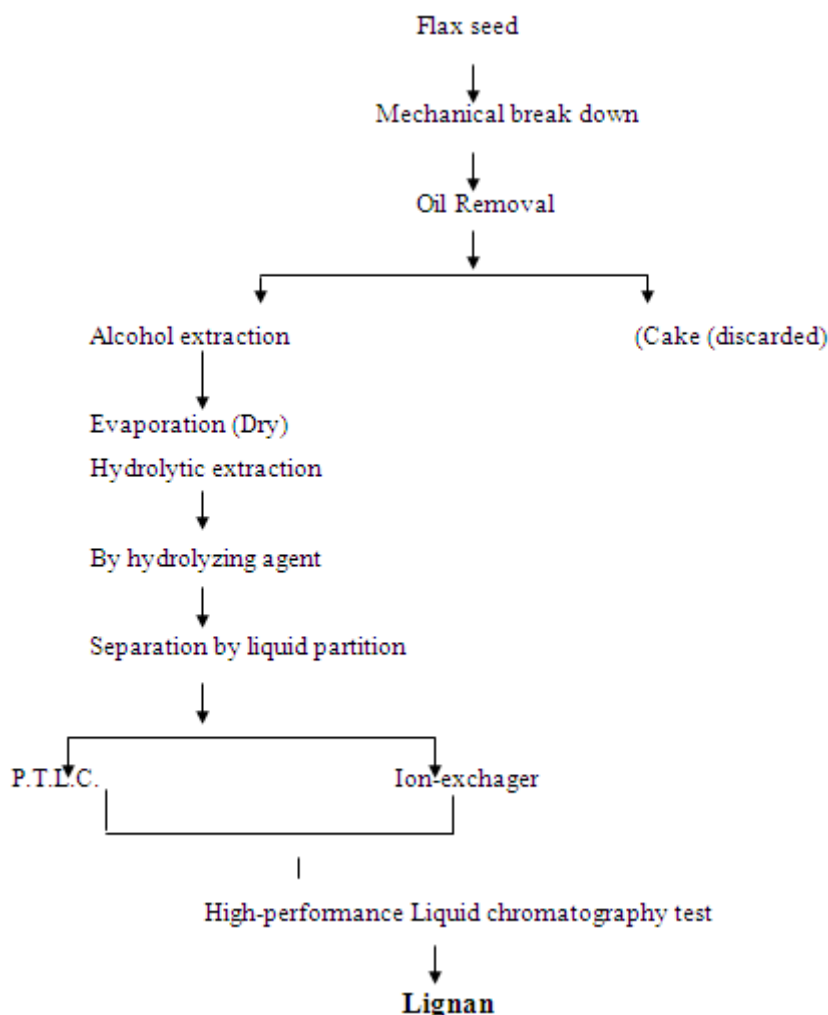
### Separated Lignan compound

A dry crude lignan dissolved in 50ml of hydrolyzing agent in vortex mixer for 24 hours. After that the suspension was filtered and the extract was evaporated and reduced pressure (45° C) to obtain the thick material with high viscosity; the pH was acidity to 3.0 by using concentrated hydrochloric acid (2M) and the sample was store at 4 C.

### Preparing test:-

According to Harborne [7] by using to separation solvent A: ethyl acetate: ethyl alcohol ratio (1:19). B. Benzene :ethyl alcohol (1:1).

By using TLC and tests under UV light at 280nm; and calculated Rf of each spot at the two separated solution.



### Liquid partition

Liquid partition method (Liquid / liquid) according to Westcott and Muir, [8] by using an ethyl acetate/ water solvent system with ratio (1:7). Ethyl acetate is particularly preferred for this purpose because of its polarity. The aqueous layer was taken, the extract was evaporated under reduced pressure(45 C) to produce dry crude.

**Purified lignan compound****Ion exchange chromatography**

A. The anion exchanger is preferably carried out in an anion exchange chromatography column in the acetate counter ion form. A-25 QAE Sephadex resin used (Pharmacia Co.) (15x 2.5 cm) and the flow rate is 1 ml/min; the 2 mls fraction was collected and the sample was tested by TLC.

B. Batch wise chromatography :-

2 ml of the crude lignan was mixed with 150 ml anion-exchanger which prepared in the acetate counter ion form; and washed with distill water after that the mixture was filtered. The filtered was evaporated under reduced pressure; the lignan was tested by TLC.

**2. Preparative Thin Layer Chromatography**

The method described by Badheka *et al.*, [9] was used. Glass plated (20x20cm) coated with silica gel thickness 1mm type (60F254). The separation solution (ethyl acetate: ethyl alcohol) was used with ratio (1:19) .

**Chemical tests for the Lignan compound**

By using the Molish's; Benedict's and fehling's test.

Ultraviolet spectroscopy test:- The test was done by the method described by (Pihlava *et al.*, [10] was used .

The visible spectrophotometer UV-160, which supply by Shimadzu Co.; and the wave length (190-400 nm) was used.

**High-Performance liquid chromatography (HPLC)**

The Pure lignan was more testing using (PHLC) according to Westcott and Muir, [12] by using ODS-reverse phase column support and an elution system under the conditions as follows:-

Column	ODS
Column length	25 cm
Flow rate	1ml/min.
Wave length	254 nm
Mobile phase	Acetic acid (100%); acetic acid (40%); methyl alcohol 60%
Rotation time	the time is obtained following the experiment

**RESULTS AND DISCUSSION**

The lignan compound (SDG) was extracted by using petroleum ether and chloroform solvent [11]. When the oil-free flaxseed meal was treated with Diowax and ethanol; obtained viscous matter and thick texture with yellowish color, the complex-compound was hydrolysis by acidity which yields the lignan. The lignan was appeared as dark spot on TLC chromatography used separation solutions (ethyl acetate: Methanol) (1:19) and (Benzene: Methanol) (1:9) (Fig. 2).

The lignan compound has Rf: 0.45 and this equal of Rf value according to Harborre [7], when used the liquid – liquid system to separate the lignan from flaxseed. The ethyl acetate and water system has weak polarity and the lignan dissolved in water and when evaporated water yellowish powder had get .[12]

The lignan is weak acid and polarity this character can used to separate by Anion exchanger chromatography by using Sephadex A-25 QAE in the two ways: first by Batch wise and the second by column. The lignan came first when washed solution (water); other compound (unless lignan) which bound resin was eluted with to the mixture (acetic acid 50%: methanol 15%). When the lignan was analyzed by TLC plate it was shown to constitute around these compounds which separated by ion-exchange chromatography

The separation and purification steps which contained lignan and tested again by P.T.L.C. (using ethyl acetate: methanol solvent and appearance that a spot is lignan compound with high purify (Fig.3). This result agreement with Badheka *et al.*, [9]. The pure lignan was tested by different reagent (Molish's; Budish and fehlinf's) and gave the positive results (Table 1). These indicate that the pure lignan is its. (Fig.4. show the Un-light test for lignan which separated by P.T.L.C method. There was one which absorbance at 200nm and this indicate for lignin compound which absorbance at this wave length [10]. (Fig.6).

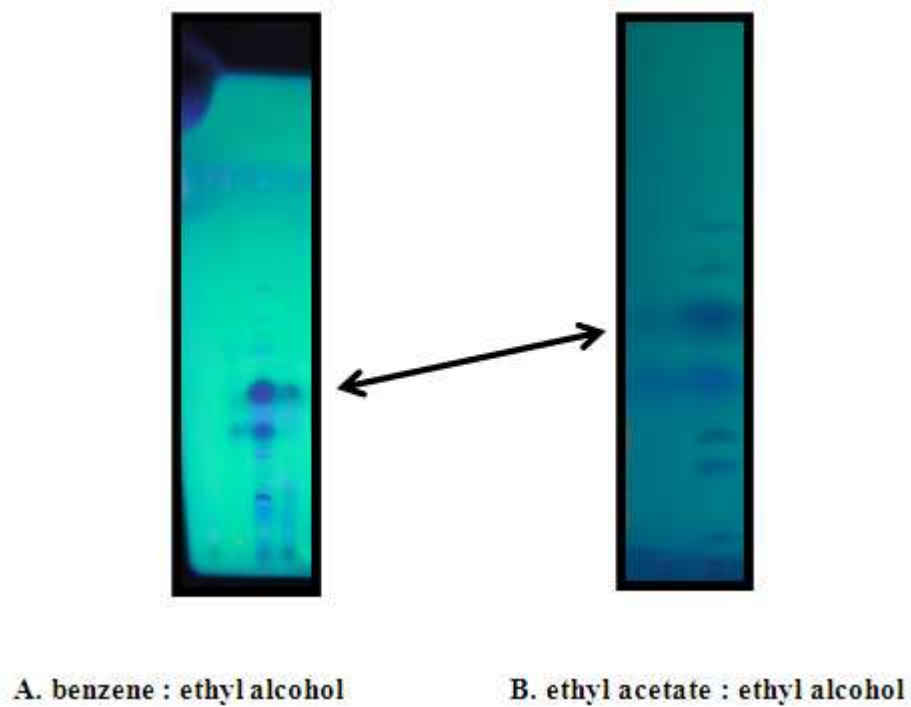


Fig (2): lignan by (TLC) primary test

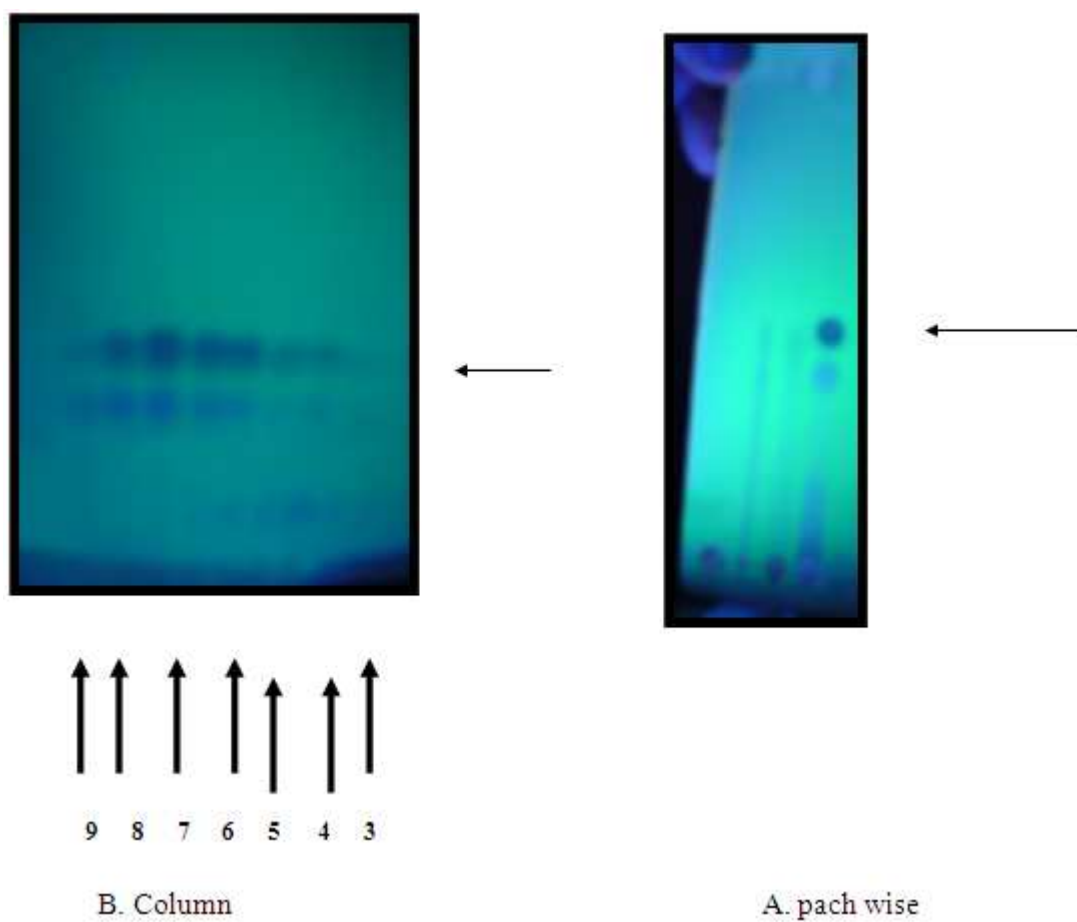


Figure (3).The separation fraction by Ion –Exchang Chromotography

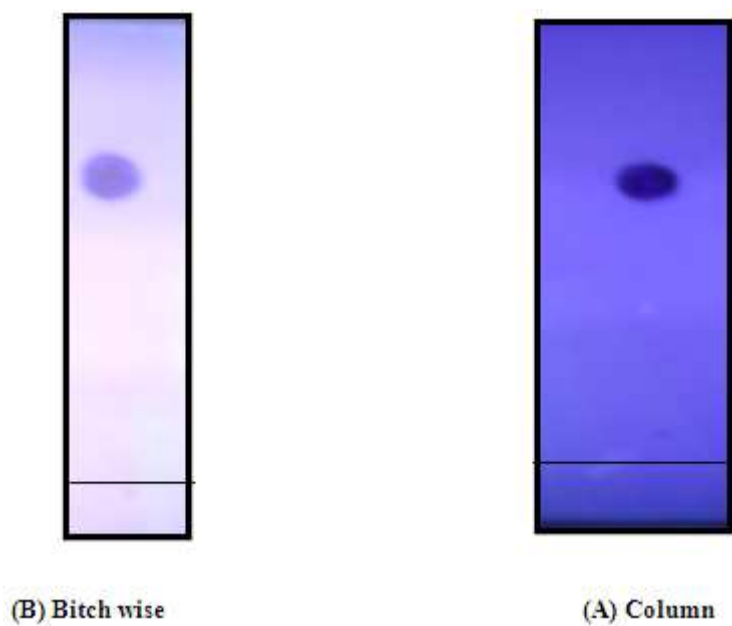


Fig (4) : Purified method by using P.T.L.C. of the lignan.

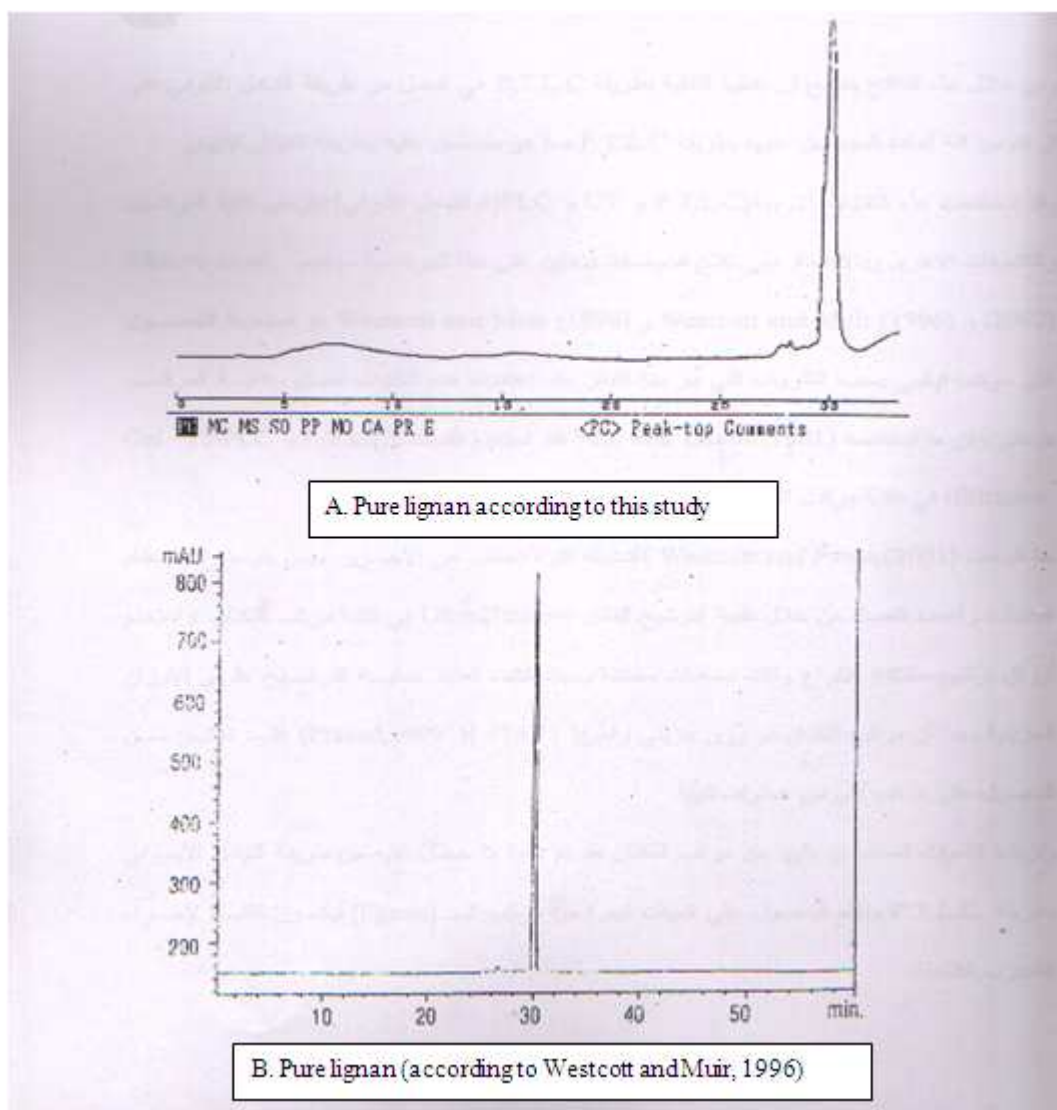


Fig. ( 5 ) : Pure Lignan compound on HPLC.

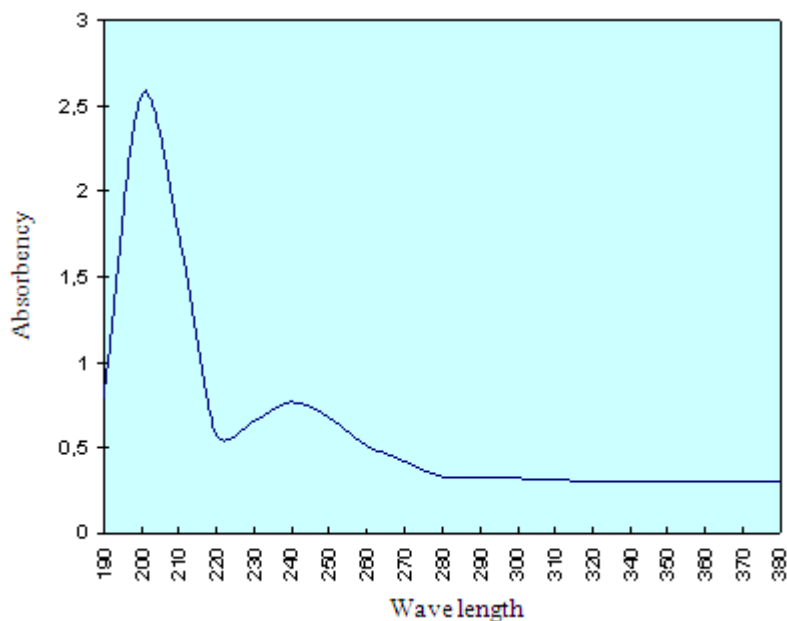


Figure ( 6 ) : Purified lignan absorbance on different wave length

Table (1) Reagent Used test for lignan compound

Test	Reagnt
+	Molish's test
+	Benedict's Test
+	Fehling's Test

Further confirmation of the results was done using HPLC, results were shown in Fig. (5). Which represented the one peak of lignan compound appearance at rotation time (29.06) min and this result agreed with the finding of [12], when they get same rotation time with a pure lignan [10].

### CONCLUSION

From the results apparent that ion-exchanger column chromatography proved is a high efficiency in purification of lignan that has been gave a proper purified amount of lignan.better than P.T.L.C. method . The difficulty to get a standard lignan compound to compared with the lignan which we purified, that was dependent on the some results which get by other researcher who work on the same compound (Lignan) [10, 13], and also compared with the results get by Paul and David, [12] who work on lignan from Podophyllum plant by using some technique (P.T.L.C; HPLC; gel filtration).

### REFERENCES

- [1] Web CJ, Flaxseed as Functional food for people and as Fees for other animals. **2003**. [www.Flax.com/12/2003](http://www.Flax.com/12/2003).
- [2] Neish AC, Coumarins, Phenylpropanes, and Lignan "in" *Plant Biochemistry* "edit" BonnerJ, and Evarner J, **1965**.
- [3] Merck Index .*The merck index of chemicals and drugs*.CD.**1999**.
- [4] Pihlava JM, Helena H, Eeva-Liisa,R and H. Veli H, Process for isolating and purifying SDG from flaxseed.*WO 02/062812 A1*.**2002**.
- [5]Thompson LU, Robb P, Serraino M, and Cheung F, *Nutr. Cancer*, **1991** 16 : 43 -52.
- [6] Rickard SE, Orcheson LJ, Seidl MM, Luyengi L, Fong HH, and Thompson LU, *J. Nutr.*, **1996**, 126:2012-2019.
- [7] Harborne JB, *Phytochemical methods, Aguide to mo dern techniques of plant analysis*. Chapman and Hall, London.**1973**.
- [8] Westcott ND, and Muir AD, Medicinal lignan from flaxseed .*PMAP Conference*.**1997**.
- [9] Westcott ND, and Muir AD, Process for extracting and purifying lignan and cinnamic acid derivatives from flaxseed .*Wo 96/30468*.**1996**.

- [10] Pilhlava JM, Helena H, Eeva-Liisa R, and Veli H, Process for isolating and Purifying SDG from flaxseed. *WO 02/062812 A1*.**2002**.
- [11] Badheka LP, Prabhu BR, and Mulchandani NB, *Phytochemistry*, **1986**, 25 (2):487- 489.
- [12] Paul MD, and David EJ, *Phytochemistry*, **1981**,20(9):2277-2280..
- [13] Westcott ND, and Muir AD, Process for extracting lignans from flaxseed. *US Patent; US005705618*.**1998**.