# Anti-ageing Activity and Identification of Bio Active Compound from the Seed of LinumUsitatissimum by GC-MS Techniques

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**ABSTRACT:**

**Objective:**Aging occurs in all living organisms and it is an inevitable process. Aging is caused due to both intrinsic and extrinsic factors. Intrinsic aging is an inevitable physiological process. Extrinsic aging occurs in addition to intrinsic aging as a result of sun and environmental damage. Activation of hyaluronidase, collagenase and elastase, leads to skin aging. By using the medicinal crop *Linumusitatissimum*were investigated to assess their skin aging properties.

**Methods:** The activity of anti- elastase, anti- collagenase and anti-hyaluronidase in ethanol extract from the seed of *Linumusitatissimum*was determined by using spectrophotometric methods. The bio-active compounds of *Lainumusitatissimum*was analyzed using GC-MS method.

**Result:**The anti-elastase assay in ethanol extract of *Linumusitatissimum*shows 70% inhibition, for anti- collagenase assay shows 73% inhibition and for anti-hyaluronidase assay shows 61% inhibition. The ethanol extracts of *Linumusitatissimum*contained the highest anti- collagenase activity.

**Conclusion:** The enzymes which are responsible for aging is inhibited by ethanol extract of *Linumusitatissimum,*suggests that it can help to restore skin elasticity and slows down the process of aging.

**INTRODUCTION:**

Aging occurs in all living organisms and it is an inevitable process. The skin protects us from microbes and the elements, helps to regulate body temperature, and permits the sensation of touch, heat and cold. Chronological aging and photoaging are the two types of skin aging [Mukheijee*et al*., 2011]. The extrinsic factor causes photoaging which includes symptoms like leathery appearance, dark or light pigmentation and deep furrows [Fisher GJ *et al*., 2002; Maity N *et al*.,2011].

The epidermis, dermis and subcutaneous tissue are the three layers in skin [Ritte L *et al*., 2002]. The outermost part of the skin is the extracellular matrix (ECM) which is made up of fibroblasts and proteins that include collagens and elastin [Fulop T *et al*., 2012]. The ECM plays an important role in the maintenance of physiological functions of the body because it is essential for growth and elasticity of the skin [Fulop T *et al*., 2012;Kurtz A Oh S.,2012]. Skin aging is directly linked with degradation of ECM and it is correlated with an increase in activity of some enzymes, which is involved in skin aging including hyaluronidase, collagenase and elastase [Maity N *et al*.,2011; Wary KK Thakkar GD.,2003; Losses JN.,2004; Maity N *et al*.,2011].

The main component of connective tissue, hair and nails is collagen and is the building blocks of skin [Mukheijee*et al*.,2011]. The elasticity, strength and flexibility of the skin is maintained by collagen. The moisture of the skin is retained by hyaluronic acid and it is also responsible for its structure and elasticity of the skin. It is involved in rapid tissue proliferation, regeneration and repair, it also facilitates the exchange of nutrients and base products [Manuskiatti W *et al*.,1996; Hsu M-F *et al*.,2009]. Hyaluronic acid is required for the organization and structural maintenance of the ECM. When collagen, elastin and hyaluronic acid level decreases it leads to loss of strength and flexibility in the skin, which further results in visible wrinkles.

*Linumusitatissimum* Linn, commonly known as Alsi belongs to the family Linaceae and it is cultivated in cooler regions throughout the world[ShwetaGokhale*et al*.,2016]. It has anticancer[Yan L *et al*.,1998; Kuijsten A *et al*.,2008], antidiabetic[Ghule AE et al.,2012], antimicrobial[Amin T *et al*.,2014] and it helps to reduce cardiovascular diseases[Gambus H *et al*.,2004;Cintra DEC *et al*.,2006]. Its oil is known as linseed oil. It is used for various medicinal

purposes. Textiles made from flax are known as linen and are used for bed sheets, underclothes and table linen [ShwetaGokhale*et al*.,2016]. This flax seed protein helps to improve immune function, lowers cholesterol, prevents tumor, has antifungal activity and it has been proved by animal studies.[Xu Y.2007; J. Agric.2010;Rabetafika, H.N *et al*.,2011]

## MATERIAL AND METHODS

### Collection of plant materials and preparation of ethanolic extracts:

The *Linumusitatissimum* seed is collected from the nearby villages in trichy and conformed its presence with the help of guide.

In a uniform powdered manner of 40 mesh size the *Linumusitatissimum*seed are grinded, before grinding the *Linumusitatissimum*seed is washed in water and dried it for a week and place it in the room temperature. The 20g of dried seed powder of*Linumusitatissimum* is dissolved in a prepared ethanolicextract(40ml) and keep it in a hot perculation for 1 hour of duration. After cooling the extract, it is filtered by using Whatmann filter paper No.42 (125mm). By using this method the undissolved products are removed which contain cellular material and some other materials. The solution which is filtered is used for the further analysis of qualitative and quantitative method.

### Chemicals and reagents:

FALGPA [N-[3-(2-furyl)acrylolyl]-leu-Gly-Pro-Ala, TES[tris(hydroxymethyl)-methyl-2-aminoethane sulponate (TES), The type I collagenase from clostridium, histolytium, ethanol, isopropanol, ninhydrine solution , cirate buffer, 4-dimethylamino benzaldehyde (DMAB), The HEPES at pH 7.5 , 4-(2-hydroxyethyl)-1-pipirzine ethanesulfonic acid, Potassium metaborate ($KBO_2$) , Hyaluronic acid , Hyaluronidase acid ,calcium chloride dihydrate , human leukocyanteelastase, N-methoxysuccinyl –Ala-Ala-Pro-Chloro.

### Anti-elastase activity determination:

For the anti-elastase activity determination, the preparation for bulk needs various concentration of *Linumusitatissimum*seed extract (20,40,60,80,100 µg/ml) was taken in the test tube and labelled it; the 100µg/ml of extract is added with 900 µg/ml of ethanol and 200 µg/ml extract is added with 800 µg/ml of ethanol and 300 µg/ml extract added with 700 µg/ml of ethanol, 400

µg/ml of extract is added with 600 µg/ml of ethanol, and 500 µg/ml of extract is added with 500 µg/ml of ethanol. For the control solution ethanol (1000µg/ml) is taken. The elastase of 500µg/ml is taken and added with 500µg/ml hepes buffer and then add 1000µg/ml of various concentration of plant sample extract and keep it in room temperature for 20 minutes then add 500µg/ml N-methoxysuccinyl-ala-ala-pro-chloro in test tubes which are incubated for further 20 minutes then check the absorbance at 540nm. The percentage inhibition was calculated as follows

Inhibition(%) = [(A control – A sample) / A control] x 100

**Anti-hyaluronidase activity determination:**

The method of finding out hyaluronidase activity is based on flurometric Morgan - Elson assay Method. 500µg/ml calcium chloride which was taken and added it with 1000µg/ml of various Concentration from the bulk solution and then add 500µg/ml hyaluronidase acid keep it for 20 minutes in a room temperature. Then add 250µg/ml of hyaluronic acid they are all kept in it for 10 minutes. The 250µg/ml of $KBO_2$ is added in a test tube. They are heated and cooled for 5 minutes then 500µg/ml DNAB is added in test tube keep it for 10 minutes incubation in room temprature check for the absorbance at 540 nm.

**Anti- collagenase activity determination:**

The method for finding out anti-collagenase activity is based on the Moore stein method which was modified by Mandal et.al. The 500µg/ml of collagenase is taken in a test tube with that 500µg/ml of TRIS buffer at the various concentration of the extract 1000 µg/ml is taken. They are all kept for 10 minutes incubation in a room temperature and then add 500µg/ml FALGPA enzyme which has been keep it in room temperature for 10 minutes then 500 µg/ml of citrate buffer solution is added and incubate it for 5 minutes in room temperature. 500µg/ml ninhydrin solution is added heat and cool it for 5 minutes and then 500µg/ml isopropanol which is added and incubate it for 5 minutes and check for the absorbance at 540nm

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**GC-MS Analysis of Flax Seed of ethanolicextrct of *Linumusitatissimum*:**

GC/MS analysis was performed at Heber Analytical Instrumentation Facility, Thiruchirapalli, for MS identification of the GC components. The column used was DB-5 (J & W Scientific, Folosm, CA) cross–linked fused silica capillary column (30 m long, 0.25 mm internal diameter) coated with polydimethylsiloxane (0.5µm film thickness). The oven temperature was programmed from 50oC for 2 min., at isothermal, then heating by 7oC/ min. to 250oC and isothermally for 10 min., at 250oC. Injector temperature was 250oC and the volume injected was 0.5 µl. Transition line and ion source temperature were 250oC and 200oC respectively. The mass spectrometer had a delay of 2 min. to avoid the solvent peak and then scanned from m/z 50 to m/z 500. Ionization energy was set at 70 eV (Fatma Mohamed El-Feky*etal*., 2016).

## RESULT AND DISCUSSION

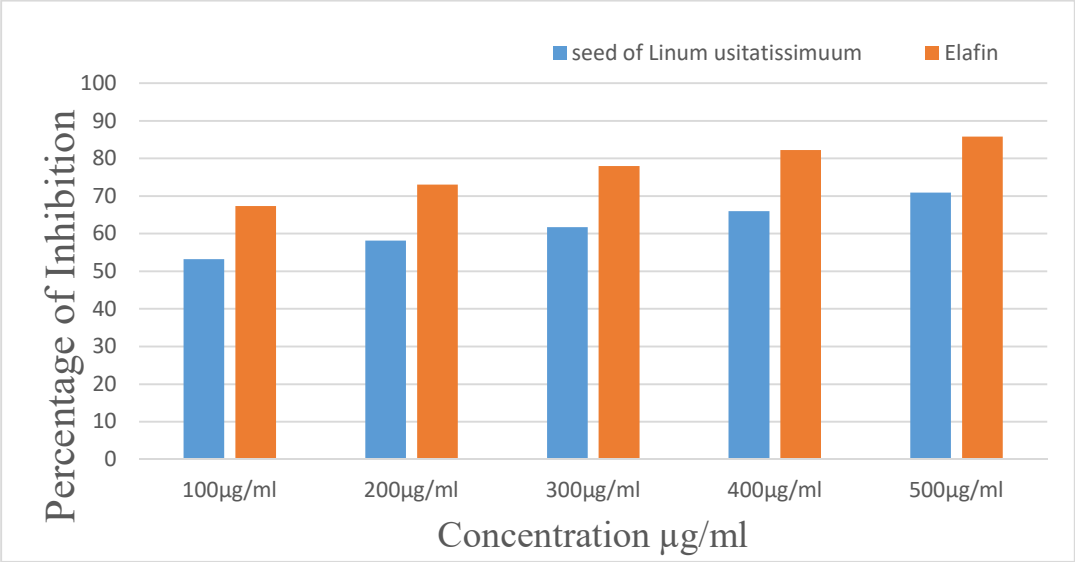
**Anti-elastase activity determination:**

In the place of connective tissues elastin is found. The elastin provides the elasticity to our skin and lungs. It is a kind of protein and it is monitorized by the enzyme called elastase. If the elastin content in our body is decreased or by exposure to UV radiation causes skin aging. Our crop *Linumusitatissimum*has provided a good firm to our skin and improve the elasticity of our skin. The ethanolic extract of seed of *Linumusitatissimum* at different varying concentrations (100µg/ml,200µg/ml,300µg/ml,400µg/ml,500µg/ml) (table 1) shows the various % inhibition concentration and the maximum inhibition concentration is showed at 100µg/ml =53.19% which is compared with the standard Elafin which show the % concentration at 100µg/ml=48.23% (Figure 1).

**TABLE 1: Determination of anti-elastase activity of seed of *Linumusitatissimum* with the standard Elafin:**

| CONCENTRATION | ANTI - ELASTASE % inhibition concentration | |
|---|---|---|
| | Seed of *Linumusitatissimum* | Elafin |
| 100µg/ml | 53.19 | 67.37 |
| 200µg/ml | 58.16 | 73.04 |
| 300µg/ml | 61.70 | 78.01 |
| 400µg/ml | 65.96 | 82.26 |
| 500µg/ml | 70.92 | 85.81 |

**Figure 1: Comparitive graph of seed *linumusitatissimum*with the standard elafin:**



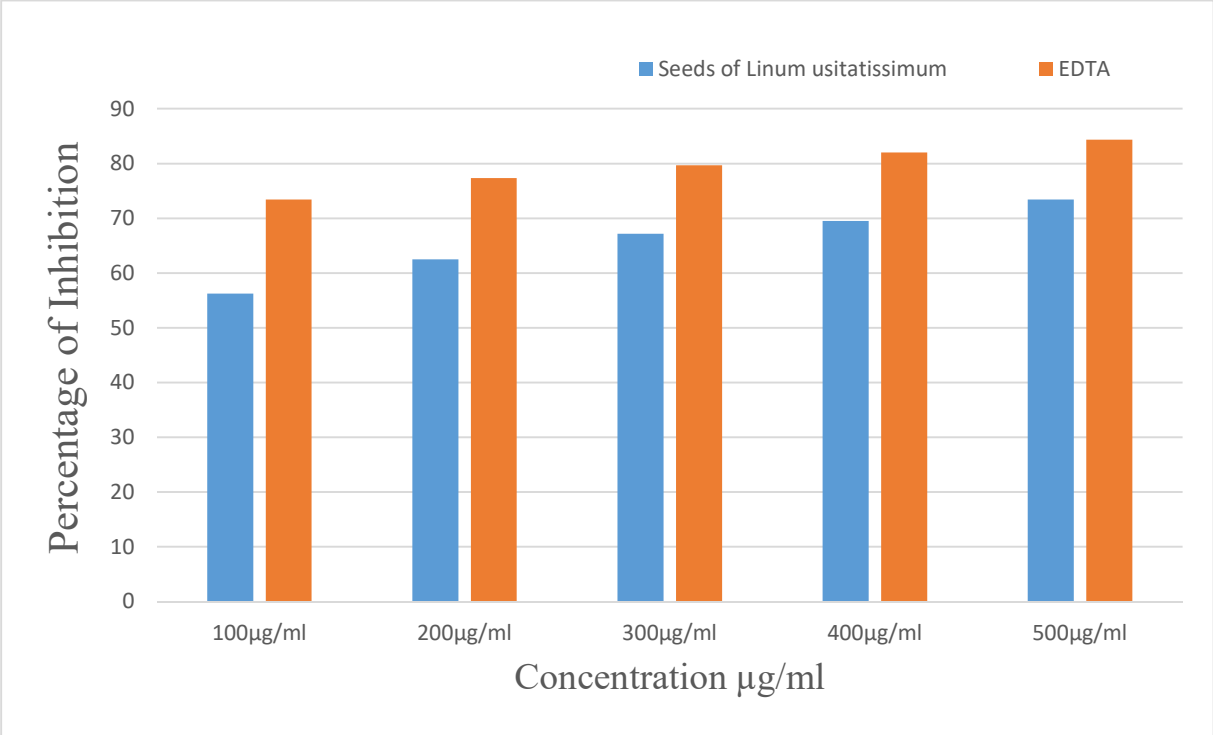
**Anti collagenase activity determination:**

The skin is the most important layer in our body and it consist of some important components like collagen with it. The collagen is declined by the presence of enzyme collagenase. If the collagenase is hindered means the aging process slowly occurs and the formation of pre-collagen fibres is delayed. The ethanolic extract of seed of Linumusitatissimum with different varying concentration (109µg/ml,200µg/ml,300µg/ml,4000µg/ml,500µg/ml) (table 2) shows the different % inhibition concentration and the maximum % inhibition concentration in the anti-collagenase activity shows at 100µg/ml=56.25% and it is compared to the standard EDTA which show the anti-collagenase (Figure 2).

**TABLE 2:Anti-collagenase activity of seed of *Linumusitatissimum* with the standard EDTA*:***

| **Concentration** | **Anti-collagenase % inhibition** | |
|---|---|---|
| | **Seed of *usitatissimuum*** | **EDTA** |
| 100µg/ml | 56.25 | 73.43 |
| 200µg/ml | 62.5 | 77.34 |
| 300µg/ml | 67.18 | 79.68 |
| 400µg/ml | 69.53 | 82.03 |
| 500µg/ml | 73.43 | 84.37 |

**Figure 2: Comparitive graph of *linumusitatissimuum*seed with the standard edta:**



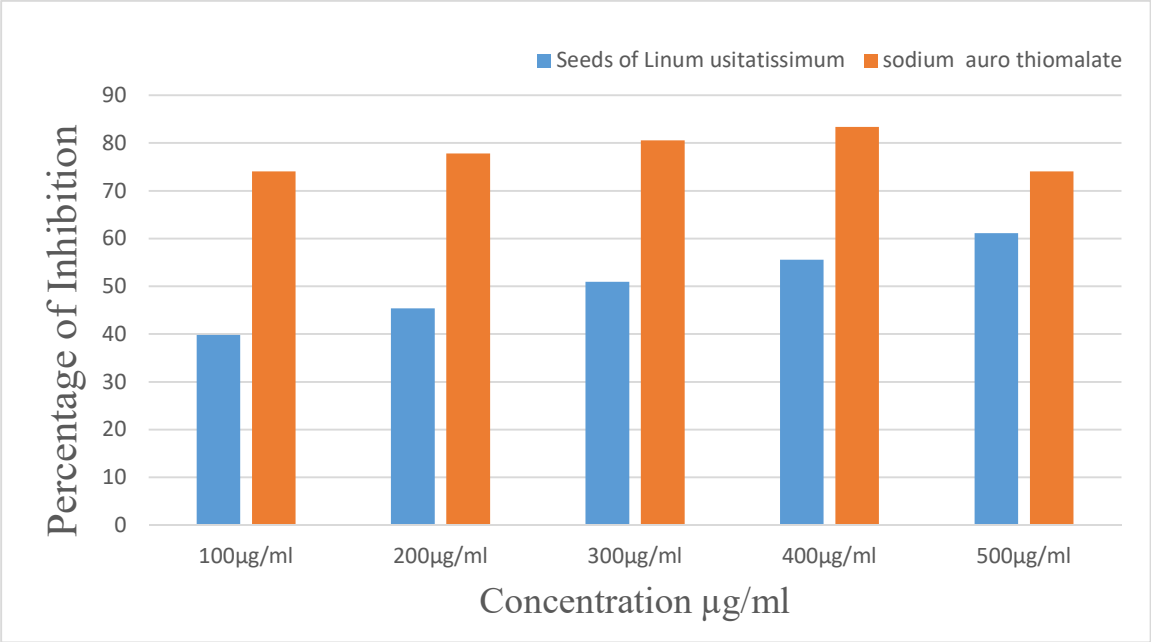

**Anti-hyaluronidase activity:**

The hyaluronidase activity is more important for our skin because it reduces the fine lines in our skin and it consists of some healing property with it. The ethanolic extract of the seed of *usitatissimuum* in the different concentrations (100µg/ml,200µg/ml,300µg/ml,400µg/ml,500µg/ml) (table ) show the different % inhibition concentration and the maximum % inhibition concentration is shown at the range of 100µg/ml=39.81% which is compared to the standard Sodium aurothiomalate. To find out the anti-hyaluronidase activity in the standard the maximum % inhibition concentration is shown at 100µg/ml= 74.07% (Figure 3).

**TABLE 3: 3Anti-hyaluronidase activity of seed of *Linus usitatissimum*compared with sodium aurothiomalate:**

| **Concentration** | **Anti-hyaluronidase activity % inhibition concentration** | |
|---|---|---|
| | **Seed of*Linumusitatissimum*** | **Sodium aurothiomalate** |
| 100µg/ml | 39.81 | 74.07 |
| 200µ/ml | 45.37 | 77.77 |
| 300µg/ml | 50.92 | 80.55 |
| 400µg/ml | 55.55 | 83.33 |
| 500µg/ml | 61.11 | 86.11 |

**Figure 3: Comparative graph on seed of *Linumusitatissimum* with the standard Sodium aurothiomalate:**




**Gas chromatography- mass spectrometry of flax seedof *linumusitatissimum*:**

BICYCLO[4.1.0]HEPT-3-ENE-2-THIOL,3,7,7-TRIMETHYL-,[1S-(1.ALPHA.,2.ALPHA.,6.ALPHA.)]-; HEPTADECANOIC ACID, METHYL ESTER; HEXADECANOIC ACID; METHYL OCTADECA-9,12-DIENOATE; (Z,Z)-6,9-CIS-3,4-EPOXY-NONADECADIENE; 9,12-OCTADECADIENOIC ACID, METHYL ESTER, (E,E)-; 7-Tetradecenal, (Z)-; Bicyclo[3.3.1]nonan-1-ol; 9-OCTADECENOIC ACID (Z)-; 1-NAPHTHALENEMETHANOL, 1,4,4A,5,6,7,8,8A-OCTAHYDRO-2,5,5,8A-TETRAMETHYL-; 1-UNDECENE-5,9-DIYNE; 1,3,5-TRIPHENYL-1,5-PENTANEDIONE; Silane, methyldiisopropoxyethoxy-; (+)-2,3-o-Benzylidene-D-threitol; Chromium, (.eta.-5-cyclopentadienyl)-(.eta.-6-toluene) (Figure 4) (Table 4).

**Figure 4: Identification of Bio active compounds from the flax seeed of *Linumusitatissimum*:**

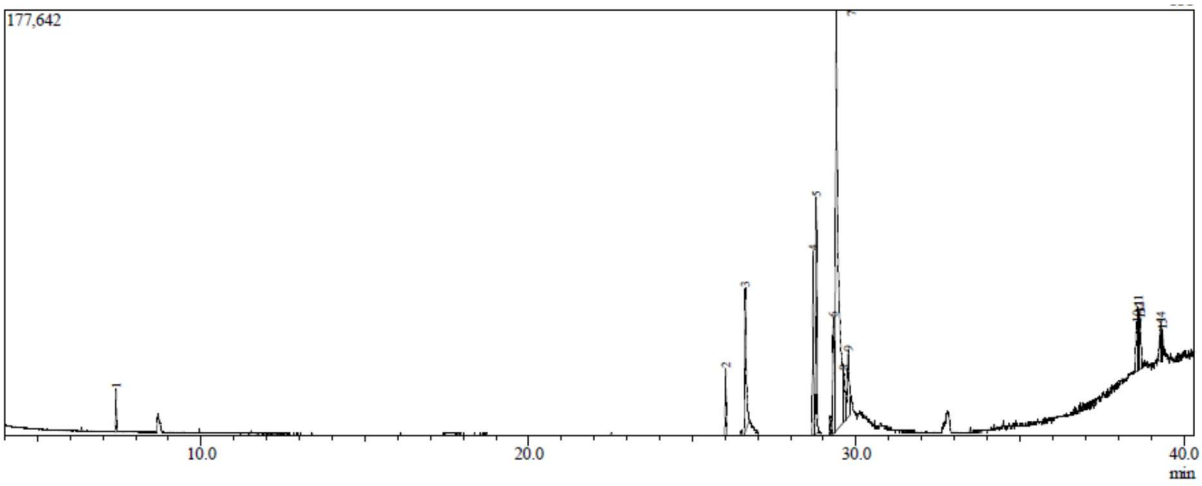
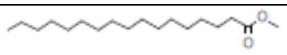
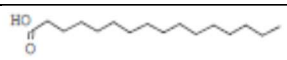
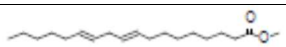
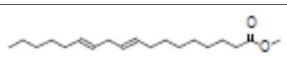
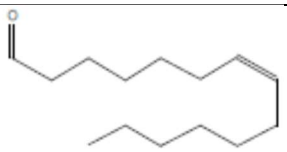
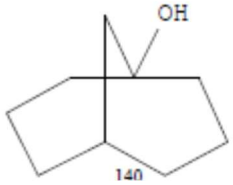
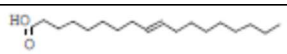
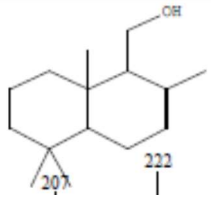
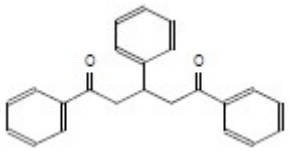
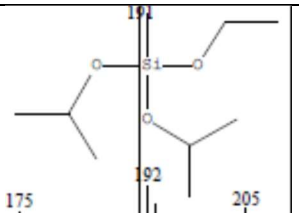
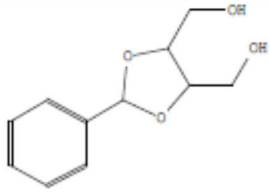
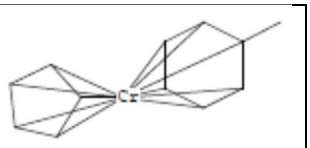



**Table 4: Identification of Bio active compounds from the flax seeed of *Linumusitatissimum*:**

| COMPOUND NAME | RETENTION | AREA% | MOLECULAR FORMULAE | MOLECULAR STRUCTURE |
|---|---|---|---|---|
| BICYCLO[4.1.0]HEPT-3-ENE-2-THIOL, 3,7,7-TRIMETHYL-, [1S-(1.ALPHA.,2.ALPHA.,6.ALPHA.)]- | 7.401 | 1.33 | $C_{10}H_{16}S$ | |
| HEPTADECANOIC ACID, METHYL ESTER | 26.012 | 2.54 | $C_{18}H_{36}O_2$ |  |
| HEXADECANOIC ACID | 26.613 | 8.14 | $C_{16}H_{32}O_2$ |  |
| METHYL OCTADECA-9,12-DIENOATE | 28.683 | 6.66 | $C_{19}H_{34}O_2$ |  |
| (Z,Z)-6,9-CIS-3,4-EPOXY-NONADECADIENE | 28.779 | 11.02 | $C_{19}H_{34}O$ | |

| | | | | |
|---|---|---|---|---|
| 9,12-OCTADECADIENOIC ACID, METHYL ESTER, (E,E)- | 29.301 | 6.25 | $C_{19}H_{34}O_2$ |  |
| 7-Tetradecenal, (Z)- | 29.392 | 43.71 | $C_{14}H_{26}O$ |  |
| Bicyclo[3.3.1]nonan-1-ol | 29.62 | 2.76 | $C_9H_{16}O$ |   |
| 9-OCTADECENOIC ACID (Z)- | 29.759 | 4.73 | $C_{18}H_{34}O_2$ |  |
| 1-NAPHTHALENEMETHANOL, 1,4,4A,5,6,7,8,8A-OCTAHYDRO-2,5,5,8A-TETRAMETHYL- | 38.565 | 3.1 | $C_{15}H_{26}O$ |   |
| 1-UNDECENE-5,9-DIYNE | 38.614 | 1.81 | $C_{11}H_{14}$ | |
| 1,3,5-TRIPHENYL-1,5-PENTANEDIONE | 38.65 | 2 | $C_{23}H_{20}O_2$ |  |
| Silane, methyldiisopropoxyethoxy- | 38.675 | 2.5 | $C_9H_{22}O_3Si$ |   |
| (+)-2,3-o-Benzylidene-D-threitol | 39.29 | 1.72 | $C_{11}H_{14}O_4$ |   |

| | | | | |
|---|---|---|---|---|
| Chromium, (.eta.-5-cyclopentadienyl)-(.eta.-6-toluene) | 39.35 | 1.75 | $C_{12}H_{13}Cr$ |  |

The previous studies suggested that the aging process mainly on skin wrinkling and identified that the skin wrinkling causes due to the body mass destruction(Kumar *et al*., 1980). Middelkoop TB *et al*., 1985 studied about theplant *Saracaasoca* are treated against menorrhagia, anti-estrogenic activity, utertonic, anti-bacterial, anti-tumour and anticancer activity. Verma A et al., 2010 studied that the natural herbs contains phytochemicals such as terpenoids polyphenols, carotenoids and some herbs which are treated against antiaging are jatamansi, alover, gensing, cucumber and honey etc.,Dhawan BN et al., 1977 studied about the anti-aging and sunscreens and they investigated the biological process of the skin aging and what are the factors responsible for aging and how to prevent the process of aging by applying the sunscreen products.

**CONCLUSION:**

The present study concluded that the *Linumusitatissimuum* has the presence of the antiaging property and it is resulted under different methods. From the qualitative test it is confirmed that the seed of *Linumusitatissimuum*inethanolic extract has shown the presence of Tannin, Steroids, Saponin, Flavonoids, Alkaloids, Terpenoids, Phlobatannin, Phenol, Glucoanthocyanin, Cardiac glycosides, Anthocyanin, Antraquinones, Glycosides, Coumarine, Emodin, Xanthoprotein, Carbohydrates and Protein content..The anti- elastase activity in the ethanolic extract of seed of *Linumusitatissimuum s*howed the maximum inhibition 5.19% which is compared to the standard elafin48.23 % at 100µg/ml. The anti-collagenase activity showed the maximum 56.25 % compared to the standard EDTA 73.44 % at 100µg/ml. The anti-hyaluronidase activity showed the maximum inhibition 39.81% compared to the standard sodium aurothiomalate74.07 % at 100µg/ml. so, it is confirmed that the seed of *Linumusitatissimuum*is helpful for the treatment anti-aging due to the presence of bioactive compounds confirmed by GCMS spectra.

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