

Evaluation of Bioactive Phytoconstituents in *Linum Usitatissimum l.* by GC-MS

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Abstract- In this study, flaxseed was subjected to identification of bioactive compounds by using Gas Chromatography-Mass Spectrum technique. Flaxseed was extracted with 99% ethanol. Extracted sample was injected, according to, the bioactive compounds are screened. The results revealed the presence of seventeen compounds of which squalene (45.27 %) and 9, 12, 15-octadecatrienoic acid (z,z,z) (24.67) were the phytoconstituents with high peak areas.

Index Terms- Bioactive Compounds, GC-MS, Flaxseed, Phytoconstituents.

I. 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 Mediterranean². Common flax was one of the first crops domesticated by man. Flax originated in India, and from its hardiness and usefulness, is generally diffused over the globe. Ancient centres of flax-growing are mountainous areas of India and China; In India flax was cultivated as a fiber crop earlier than cotton. As early as in the 4th or 5th millennium B.C. flax was cultivated for its fiber in Mesopotamia, Assyria and Egypt. Wild narrow-leaved flax and semi-cultured procumbent flaxes grow in Transcaucasia. Many monuments of Ancient Egypt reflect cultivation of flax and spinning and weaving of its fibres. In Russia flax has been cultivated since the birth of the Russian nation³.

Annual: stem-cylindric, erect, simple below, 0.6-1.2 m, often solitary corymbosely branched above: leaves narrow sub-3-nerved, linear or lanceolate without stipular glands: flowers 2.5cm diam, in broad cymes: sepals ovate, acuminate, 3-nerved, glandular, margins ciliate or not, petals blue, styles quite free: stigmas linear-clavate: fruit--is a rounded capsule about one – fourth inch or more in diam, surrounded by the persistent sepals. It possesses 10 locules, each with one seed. The seed is oval, lenticular, and pointed at one end. Its length varies between 4 and 6 mm. and breadth between 2 and 3 mm. Each capsule contains ten seeds⁵.

Flaxseed (*Linum usitatissimum* L.) Diet has been suggested to be the most important environmental factor. Flaxseed (FS), a whole grain used as a nutritional supplement, has gained popularity because it is a rich source of natural antioxidants. FS has high concentrations of omega-3 fatty acids and lignans. Omega-3 fatty acids reduce inflammation and may be helpful in treating a variety of autoimmune diseases¹. 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”⁴. Hence, the main objectivity of this study to identify the phytoconstituents to do the further research parts in cancer

II. RESEARCH ELABORATIONS

Extraction procedure: seeds of flaxseeds were bought from Nilgris market, Coimbatore. 10gm powdered flaxseed was soaked in 20ml of Absolute alcohol overnight and then filtered through a Whatman® No. 41 filter paper (pore size 20 - 25_μm) along with 2gm Sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and was concentrated to 1ml. The extract contains both polar and non-polar phytoconstituents.

Gas Chromatography–Mass Spectrometry (GC/MS) Analysis

GC/MS analysis of this extract was performed using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) equipped with a Elite-1 fused silica capillary column (30 m × 0.25 mm ID. ×1 μMdf, composed of 100% Dimethyl poly siloxane). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min. and an injection volume of 2 μl was employed (split ratio of 10:1). Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative percentages were calculated.

Characterisation of Compounds

Interpretation on mass-spectrum GC-MS was conducted using the database of National institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

III. RESULT AND DISCUSSION

Table I: PHYTOCOMPONENTS IN ALCOHOL EXTRACT

No	RT	Name of the compound	Molecular formula	MW	Peak Area %
1.	3.59	Butane ,1,1-diethoxy -2-methyl-	C ₉ H ₂₀ O ₂	160	0.45
2.	4.02	Hexanoic acid,ethyl ester	C ₈ H ₁₆ O ₂	144	0.25
3.	5.00	Propane , 1,1,3-triethoxy-	C ₉ H ₂₀ O ₃	176	0.17
4.	7.45	2-Furancarboxaldehyde,5-(hydroxymethyl)-	C ₆ H ₆ O ₃	126	2.72
5	8.52	2-methoxy-4-venylphenol	C ₉ H ₁₀ O ₃	150	0.32
6	9.61	Cyclohexane,1,2,4-trimethoxy-,stereoismer	C ₉ H ₁₈ O ₃	174	0.49
7	10.29	Sucrose	C ₁₂ H ₂₂ O ₁₁	342	9.80
8.	11.78	8-Azabicyclol [3,2,1]ocatane,8-acetyl-	C ₉ H ₁₅ NO	153	2.18
9.	17.38	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	7.44
10	17.76	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	0.85
11	20.12	Palmidrol	C ₁₈ H ₃₇ NO	299	8.94
12	20.22	9,12,15-octadecatrienoic acid,(z,z,z)-	C ₁₈ H ₃₀ O ₂	278	24.67
13	20.52	Oleic acid	C ₁₈ H ₃₄ O ₂	282	10.16
14	22.33	Hexadecanal,2-Methyl-	C ₁₇ H ₃₄ O	254	1.69
15	22.93	Pyrolidine,1-(1-oxo-7,10-hexadecadienyl)-	C ₂₀ H ₃₅ NO	305	17.60
16	25.06	1-Monolinoleoglycerol trimethylsithyl ether	C ₂₇ H ₅₄ O ₄ Si ₂	498	4.56
17	31.24	Squalene	C ₃₀ H ₅₀	410	45.27

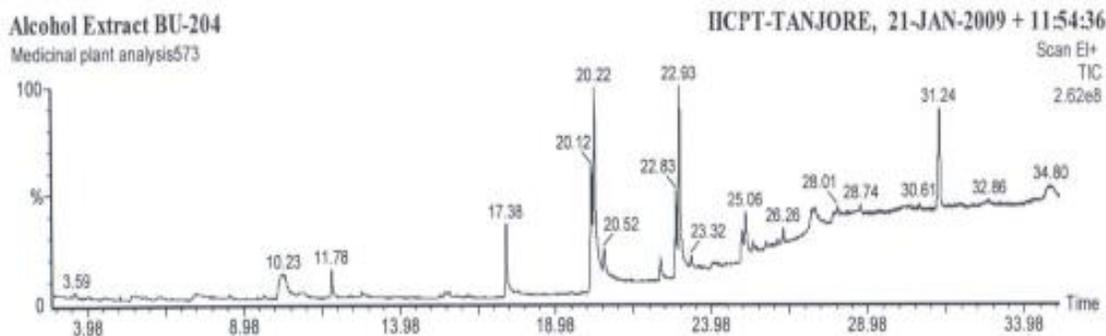


Figure 1: Phytocomponents of alcoholic extract of flaxseed

The studies on the active principles in the flaxseed ethanolic extract by GC-MS analysis clearly showed the presence of seventeen compounds (Tab-1). The active principles with their retention time (RT), molecular formula, molecular weight (MW), and concentration (peak area%) are presented in Table-1. The GC-MS chromatogram of the seventeen peak of the compounds detected was shown in Figure-1. Chromatogram GC-MS analysis of the ethanol extract of flaxseed have been showed the presence of 5 major peaks and the components corresponding to the peaks were determined.

IV. DISCUSSION

The spectral analysis of the ethanol extract of flaxseed revealed the presence of compounds by forming 5 major peaks

were squalene (45.27%), 9, 12, 15, octadecatrienoic acid,(z,z,z)- (24.6%), pyrrolidine, 1-(1-oxo-7,10-hexadecadienyl)- (17.60), oleic acid (10.16%) and sucrose (9.80 %) respectively. The triterpene has also been found to have protective activity against several carcinogens. Substances related to squalene, including β -carotene, coenzyme Q10 (ubiquinone) and vitamins A, E, and K¹⁰. The primary therapeutic use of squalene currently is as an adjunctive therapy in a variety of cancers. Although epidemiological, experimental and animal evidence suggests anti-cancer properties, to date no human trials have been conducted to verify the role this nutrient might have in cancer therapy regimens^{9,7}. oleic acid (omega-9) is found in animal and vegetable oils and is the major component that is responsible for health benefits of the Mediterranean diet, rich in vegetables and fruits. Although researchers were aware that a Mediterranean diet can reduce the risk of breast cancer and other illnesses such as

heart disease, until now they did not know how⁶. In this study, flaxseed was extracted with absolute alcohol and subjected to screening of bioactive compounds by Gas chromatography- Mass spectrum technique. The presence of 5 major peak have shows the anticancer properties.

V. CONCLUSION

This study denotes the various phytochemicals in flaxseed and it has some anticancer properties. Further studies should be needed to study anticancer properties in breast cancer.

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