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Advance Phytochemical Screening of Active Phytocontents of Linum Usitatissimum and Guizotia Abyssinica Plant Seeds in Spectrometry a Study of Comparative Properties

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Abstract: Many plant Species used in the treatment of different diseases. Plant derived active compound have played an important role in the development of clinically useful agents. Guizotia abyssinica and Linum usitatissimum plant seeds are used for many disease treatments. Aim of the present study is investigate the phytochemical analysis of Methanol, petroleum ether, Chloroform and Acetone extracts of linum usitatissimum and Guizotia abyssinica plants by spectrometry like UV, IR, NMR and Mass spectrometry. Qualitative analysis of phytochemical Screening reveals the presence of Phenol, Saponins, Alkaloids, Protein and Carbohydrates. Current research describes a simple, effective and reproducible Comparative phytochemical analysis of natural seeds.

Keywords:- *Medicinal plants, Phytochemicals, Guizotia abyssinica, Linum usitatissimum, Spectrometry, Antioxidant activity.*

I. INTRODUCTION

Plants produce an amazing variety of metabolites that are gaining importance for their therapeutic and biotechnological applications. Plants have been an important Source of medicine for thousands of years. Plants are the source of many modern medicine. Phytochemicals are responsible for the healing properties of plants. Plants turn out many secondary metabolites together with flavonoids, Alkaloids, Steroids, Saponins, terpenoids and glycosides to safeguard themselves from the attack of present infectious agent, insects, pest and environmental stresses.[1]

Niger (Guizotia abyssinica) is an oil seed plant cultivated for over 5000 years.It is widely grown in Southern India and Ethiopia. In India, it is cultivated on the slopes of hills and plains along the coasts of Madhya Pradesh, Chhattisgarh, Odessa, Maharashtra, Bihar, Karnataka, and West Bengal. G. abyssinica dicotyledonus plant, medium to fine branches, growing up to 2 m high. The plant grows very well in poorly drained, heavy clay soils. An important feature of this plant is that it provides good seed yield even under poor growing conditions. Niger is heavily cultivated for the extraction of Oil used for soap, lighting, lubrication and used as biodiesel. Niger oil absorbs the fragrance of flowers used as a base oil in the perfume industry. The plant is used in various Indian communities for the treatment of rheumatism, rheumatoid arthritis and infectious diseases.[2]

Flax (Linum usitatissimum) plant growing to one m tall. The seeds are oval, 2.5-9.5 cm long and 1-3.5 cm. thin shiny in experienced depilatory with a black and a brief stalk regarding are 1-1.8 cm long seeds of Linum usitatisamum plant are used medication for treatment of Rheumatism, Dyspepsia, stomach upset, Dysmenorrheal, Diabetes, Cardiovascular disease, Cancer, Expelling disorders, Skin diseases, Trauma symptom and has sedative and antiviral properties. The seeds and alternative Components of Linum usitatissimum plant periwinkle exhibit inhibitor properties. Therefore, phenoplast compounds have chemical reaction properties that act as reducing agents, chemical element donors.It's multiple applications in foods, cosmetics and Pharmaceutical industries. Besides inhibitor activity, these compounds exhibit antiallergic, medicinal drug, antimicrobial antithrombotic cardio protecting and vasodilatory effects.[3]

Phytochemicals are basically divided into two groups of Primary and secondary metabolites based on the activity of plant metabolism. Primary or basic metabolites include regular carbohydrates, amino acids, proteins and chlorophyll while secondary metabolites include alkaloids, saponins, Steroids, flavonoids, tannins and more.[4]

II. MATERIALS AND METHODS

A. Collection of Samples:

Guizotia abyssinica and Linum usitatissimum are collected from forest region of Betul, M.P., India were Collected in the winter season and summer season. The plants calibrated taxonomically and was preserved for extraction.

B. Preparation of Solvent Extracts of plants

Guizotia abyssinica seeds were properly cleaned with running water then properly removed with purified water. The Seeds dried for 5 days at ambient temperature for shade. Second, dried seeds were coarsely used with a mortar and pestle and then a mechanical blender was used to ground them further. 30gm 340 ml of organic Solution of Methanol & D.W. were collected from the sample extraction at Soxhlet. The extraction was completed in 8 days at 65°c. In order to form a paste, extract were then

evaporated at 45^0C and further transfer to sterile and refrigerated once used. [5][6]

Linum usitatissimum seeds were properly washed with running water then purified water. The Seeds dried and crushed to urge powder. Dried powder of seeds 50gm was hot extracted with 500 cc fuel exploitation soxhlet instrumentation. [7]The Soxheletion at 30^0C was done for one week to obtain extract. The extract was evaporated in water bath at 70^0C to obtain Crude for phytochemical analysis. Once the entire evaporation, the load of the extracts was recorded and so labeled.[8] The extractions stored separately at 4^0C in air tight battle. After dried powder 50gm cold extracted with 200ml Petroleum Ether using rotary shaker and were incubated for 1 week at 25°C at least 5 times vibration per day.[9]The extract were filtered exploitation textile material and volatilized exploitation rotary distillation instrumentation, this extract dried in 50^0C oven for 24 hours and finally kept at 4^0C temperature.[10]

C. Identification tests for Phytochemical Constituents.

Phytochemical analysis was performed to determine the presence of bioactive Compounds like carbohydrates, proteins, starch, amino acids, steroids, glycosides, flavonoids, alkaloids, tanning, Saponins, Phenols and resins by the following procedure [11]

a) Test for Alkaloids
5ml of the prepared extracts were volatilized to standing. The residue was taken in 5 ml of acid, saturated with chemical compound and filtered. The filtrate was one by one tested with following reagents:

- **Wagner's Test**
  To few ml of each of the sample solution, Wagner's reagent (iodine in potassium iodide) was added, which resulted in the formation of reddish brown precipitate indicating the presence of alkaloids.

- **Mayer's Test**
  To 1ml of each of the sample solution few drops of Mayer's reagent (Potassium mercuric chloride solution) was added. Formation of cream white precipitate indicates the presence of alkaloids.

b) Test For Phenols.
To the crude extract 2ml of 22 metal chloride resolution was extract and black coloration was firm for the presence of phenols.

c) Test for flavonoid
To 4cc of extract add 1.5cc 50% methanol solution. The solution was heat and metal chemical element was further to this solution, 5-6 drops of centered HCl was further, red color made up our minds for flavonoids and Orange color for flavones.

d) Test for Quinones
About 0.5 gm of plant extract was taken and extra 1 c.c. of extract and 1cc of con. H_2SO_4 was extract formation of red color shows the presence of quinones. One drop of ethanol take a look at resolution is placed on a filter paper, followed by one drop of 0.2% ethanolic phenylacetonitrile resolution and one drop of 0.1 N hydroxide. A positive response is indicated by the appearance of a blue or violet stain edged by a yellow ring.

e) Test for terpenoids (Salkowski Test)
5cc of each extract was mixed during a try of cc of Chloroform and con. H_2SO_4 (3ml) was strictly extra to create a layer. A brown coloration of the bury face was intentional to indicate positive results for the presence of terpenoids.

f) Test for Tannins:
To 5ml of extract, few drops of 5% ferric chloride solution were added. The appearance of violet indicates the presence of Saponins.

g) Test for saponins
To 0.5 ml of filtrate, added 5ml of distilled water and shaken vigorously for a stable persistence froth. Frothing which persisted on warming indicates the presence of saponins.

h) Test for Steroids.
To a 3 cc of extract add a 3 cc Chloroform and 3 cc of con. H_2SO_4 shake well; chloroform 1 layer show chromatic color light.

i) Test for Fatty Acids.
About 0.5 ml of extract was mixed 5 ml of ether. The extract was allowed to evaporate, on filter paper and dried. The appearance of transparence on filter paper indicates the presence of fatty acids.

j) Test For Carbohydrates
For 2ml test solution added 2 drop of the molisch's reagents (a solution of α-napthol in 95% ethanol). Therefore solution is then poured slowly in to a tube containing 2 cc of center red vitrial So 2 layers kind. Purple to ruby violet color at the junction of 2 layers indicates the presence of macromolecule.

k) Test for Glycosides
To the solution of the extract add glacial carboxylic acid, few drops baseball metal Chloride and 1cc red vitriol further and determined for a brown coloration at the junction of two layers and additionally the bluish in experienced colorize the upper layer.

l) Test for Proteins.
a) Xanthoprotein Test
The extracts are treated with a few drops of Con.HNO_3; the yellow color indicates the presence of protein

m) Check Amino Acids.
   a) Nanhydrin Test
      In 1ml of boiled sample with a 0.1% acetone solution of ninhydrin, the appearance of pink indicates the presence of amino acids.

III. RESULTS AND DISCUSSION

- **Observations:**

S.N.	Phytochemical Constituents	Guizotia abyssinica	Linum usitatissimum
1.	Alkaloids	+ve	+ve
2.	Flavonoids	+ve	+ve
3.	Phenolics	+ve	+ve
4.	Terpenoids	+ve	+ve
5.	Saponins	+ve	+ve
6.	Fatty acids	+ve	+ve
7.	Glycosides	+ve	+ve
8.	Carbohydrates	-ve	-ve
9.	Proteins	+ve	+ve
10.	Amino Acids	+ve	+ve

Table 1: Phytochemical Screening of extract of Guizotia abyssinica and Linum usitatissimum

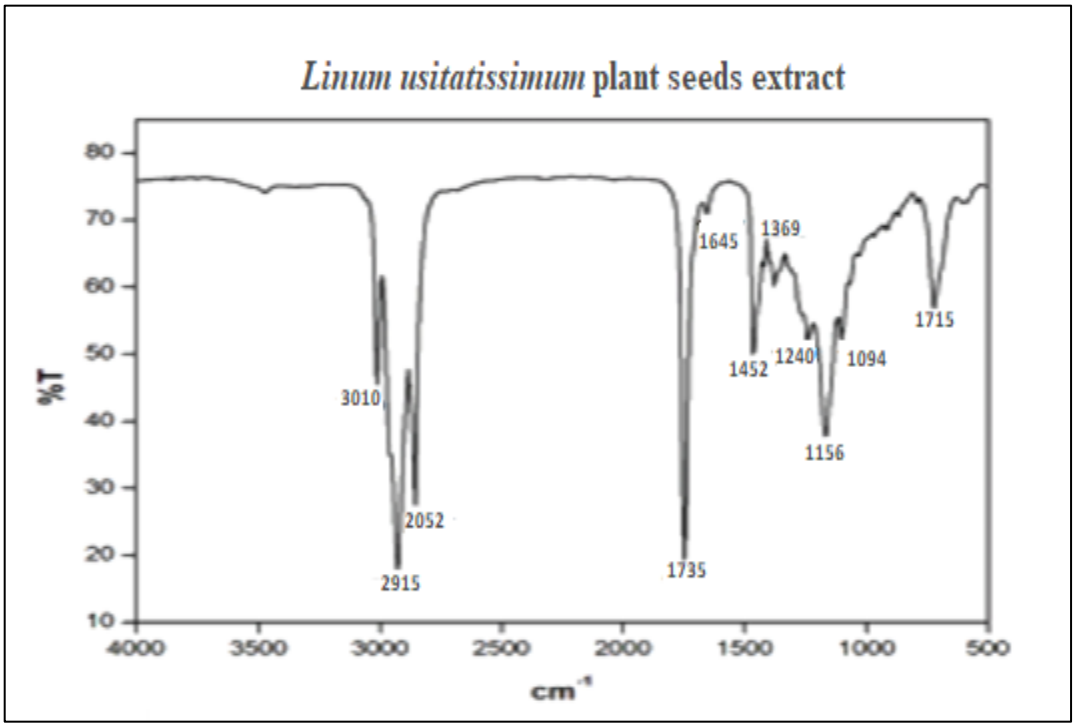

Fig.1: FTIR Spectrum of *Linum usitatissimum* plant seeds extract

The FTIR analysis confirms the presence of unsaturated fatty acid ester due to the presence of bands of 3010 cm , 1715 cm , 1645 cm and 1240 cm , these and the other bands are also reported in other research. The band at 1735 cm refers to the stretching of the ester carbonyl and the band at 1240 cm to the stretching of the CO bond. Moreover, the bands at 3010 cm and 1645 cm are attributed to the double bonds present in the chains as can be seen in Figure 1.

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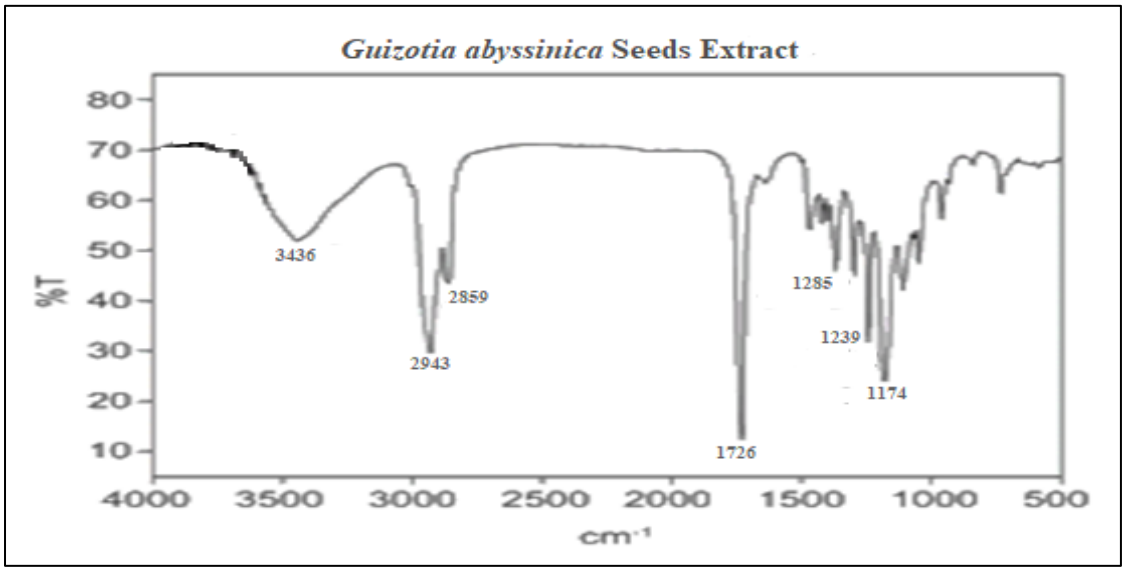

Fig. 2: FTIR Spectrum of *Guizotia abyssinica* plant seeds extract

The FTIR analysis confirms the presence of unsaturated fatty acid ester due to the presence of bands of 3436 cm , 1174 cm , 1239 cm and 1285 cm , these and the other bands are also reported in other research. The band at 1726 cm refers to the stretching of the ester carbonyl and the band at 1285 cm to the stretching of the CO bond. Moreover, the bands at 3436 cm is attributed to the double bonds present in the chains as can be seen in Figure 2.

^{1}H-NMR spectrum of Linum usitatissimum and *Guizotia abyssinica* seed extract

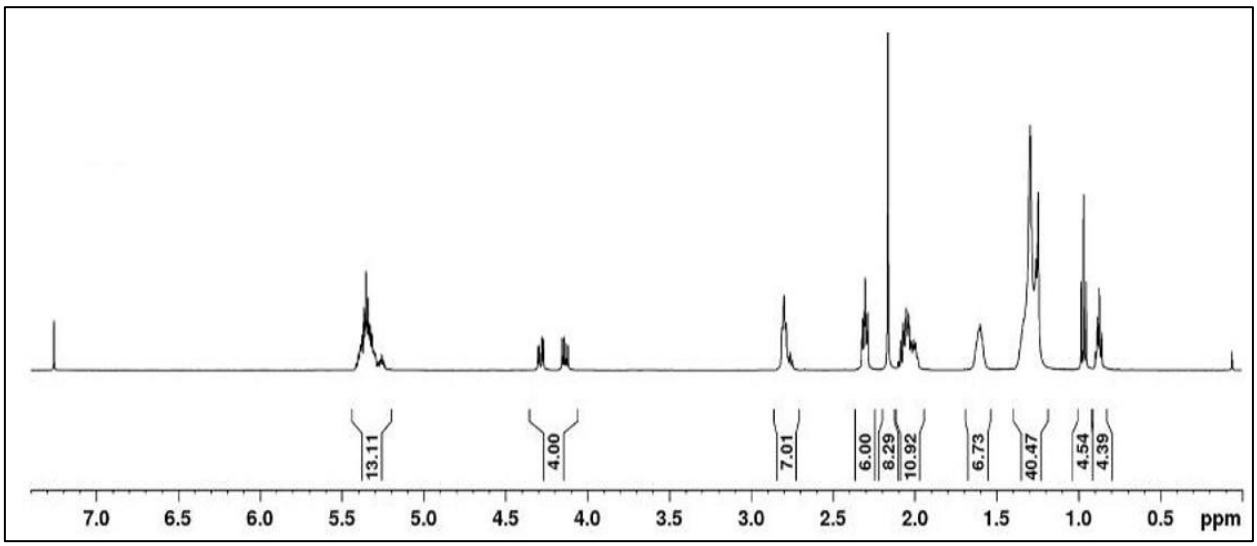

Fig. 3 : ^{1}H-NMR spectrum of *Linum usitatissimum* seed extract at 400 MHz in $CDCl_3$

The 1H-NMR spectra of *Linum usitatissimum* seed extract , shown in Figure 3 , show the number of protons at each chemical shift with different acyl groups. The spectral signals are attributed to the presence of protons in triglycerol and the area of each chemical shift is related to that of *Linum usitatissimum* seed extract before and after enrichment. It can be seen that the proton count at each chemical shift is the same except for the unsaturated region (5.2–5.3 ppm) and the chemical shift at 2.3. ppm, which indicated that the fatty acid composition of *Linum usitatissimum* seed extract enrichment is similar. Similar observations are observed in section 3.1, as described in the Materials and Methods section, fatty acid composition calculated using 1 second H-NMR spectra (Andrade et al., 2011) and linolenic acid. The iodine value was calculated by 1H-NMR analysis with the Hunas method (IV = 10.54 + 13.39 *% olefinic protons in the lineages). IS. oil), and turns out to be 169.3 grams of I2 / 100 grams of *Linum usitatissimum* seed extract . from the results of the study. Reported in section 3.6. Therefore, enriched *Linum usitatissimum* seed extract can be a potential supplement for food, pharmaceutical and nutraceutical applications.

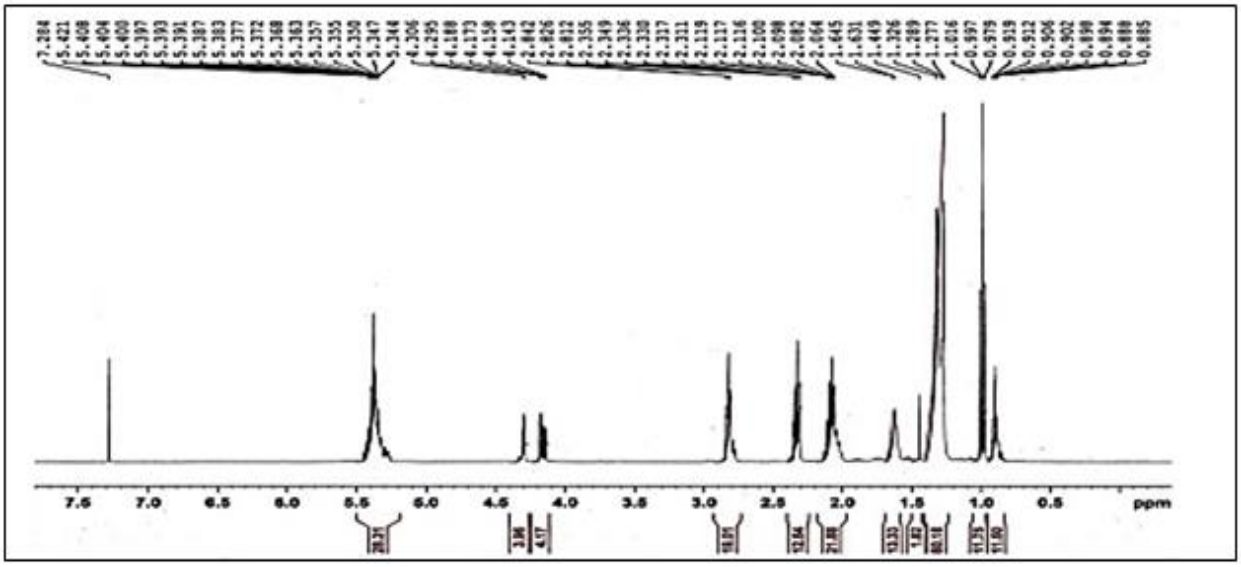

Fig. 4: ^{1}H-NMR spectrum of *Linum usitatissimum* seed extract at 400 MHz in $CDCl_3$

The 1H-NMR spectra of *Guizotia abyssinica seed extract*, shown in Figure 4, show the number of protons at each chemical shift with different acyl groups. The key signals were observed as triplets at 0.87 and 0.98 ppm belonging to terminal methyl groups, multiplets at 1.26 and 1.32 ppm due to the methylene protons of the carbon chain, multiplets at 1.64 ppm assigned to protons β-carbonylmethylene, at 2.08 The ppm corresponding to the methylene proton of the emerging multiplet allyl groups, the back at 2.32 ppm due to methylene in the carbonyl groups α, another back at 2.81 ppm characterized by a diallylmethylene proton , the two doublets centered at 4.16 and 4.31 ppm which are related to glycerylmethylene. 5.34 ppm overlap each other at 5.38 ppm belonging to glycerylmethine and oleic proton respectively. It is worth noting here that the absence of any signal in the 3.5 to 4 ppm region indicates that the *Guizotia abyssinica* seed extract does not contain diglycerides and monoglycerides in quantities detectable by the 1H-NMR technique. Furthermore, a good agreement was observed between each iodine value and the *Guizotia abyssinica* seed extract composition in the fatty acids on the hand and the proton NMR data of the *Guizotia abyssinica* seed extract on the other hand.

This study has discovered the presence of healthful chemical constituents. Phytochemical experiments are expected to assist on the accurate identification of high quality materials where plant chemistry differs between different species. All solvents namely Methanol, Ethanol, Petroleum ether, Chloroform and seed water, natural leaf and callus produce highly variable effects on the presence of nutrients bioactive substances such as alkaloids, flavonoids, Terpenoids.[12][13]

The selection of Crude plant extracts for screening programs has the potential of being heaps of thriving in initial steps than the screening of pure compounds isolated from natural merchandise. The plant extracts provision of secondary metabolites i.e. alkaloids, flavonoids, terpenoids, tannins, Glycosides, Steroids etc. Plant extract are known to be effective on steroids. Which are very important compounds because they are related to compounds such as sex hormones and it has been reported that steroids have Cardiotonic activities and antibacterial properties.[14]

The phytochemical analysis of the Guizotia abyssinica and Linum usitatissimum are important and has in every business interest analysis institutes and medicine prescribed medication Companies for the manufacturing of the new drugs for treatment of various diseases.[15]

IV. CONCLUSION

It can be concluded from the present study that Guizotia abyssinica and linum usitatissimum contains a major secondary bioactive compounds such as Alkaloid's, flavonoids, terpenoids, tannins, Glycosides are commercial value and can lead to great interest in phyto pharmaceuticals.[16] Healthful plant plays a major role in preventing various diseases.[17] The medicinal drug, medicament, antioxidant, anti-abortifiecient of the various elements of plants is because of the presence of the on prime of mentioned secondary metabolites.[18] Highly variable effects on the presence of nutrients bioactive substances India recently increased research in Traditional Herbal Medicines following scientific confirmation of their effectiveness in treating conditions for which they were traditionally prescribed. The present study provides proof that solvent extract of Guizotia abyssinica and Linum usitatissimum is contains medicinally necessary bioactive compounds and this justifies the utilization of plant species as ancient medication for treatment of various diseases.[19][20] Additional purification, identification and characterization of the bio active chemical constituent's Compounds would be our priority in future Studies.

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