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ANTIOXIDANT, ANTIDIABETIC POTENTIAL AND QUANTIFICATION OF LUPEOLL IN METHANOLIC EXTRACT OF *ANETHUM SOWA* LINN. (SEED)

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Abstract

Anethum sowa is widely used traditional medicine in many countries and in formulations of drugs. A simple, rapid, selective and quantitative HPTLC method has been developed for determination of Lupeol in *Anethum sowa* and a scientific evaluation of its traditional claims viz. anti oxidant and anti diabetic activity. The methanolic extract of *Anethum sowa* samples were applied on TLC aluminium pre coated plate with Silica gel60 GF-254 and developed using Toluene:Ethyl acetate: Formic acid (5:3:0.8v/v) as a mobile phase. Spectrophotometric evaluation was performed at 400 nm. Lupeol was resolved at R_f 0.58 ± 0.03 by pre chromatographic derivatization with anisyldehyde sulphuric acid. Anti oxidant capacity, reducing power of the species increases linearly with concentration, similar to standards. The IC_{50} value for the *in vitro* DPPH method was 1.22 ± 0.411 mg/ml. *In vitro* anti diabetic activity was analyzed by starch-iodine assay and 3, 5-dinitrosalicylic acid method. In the starch-Iodine assay IC_{50} of methanolic extract was found to be 252.9 ± 0.05 µg/ml and 0.236 ± 0.04 mg/ml for the DNS method.

Keywords: *Anethum sowa,* linolenic acid, DPPH; reducing power; total antioxidant capacity, α- amylase.

1. Introduction

Anethum sowa L. or dill, belonging to *Apiaceae* (*Umbelliferae*) family, is an annual aromatic herb known for culinary and medicinal use since ancient times. It is an annual herb with pinnately divided leaves. The plant grows up to a height of 150cm, stem is round and 2-5 branches arise from the base of stem and grow along with the main stem. Flowers are yellow in colour. The seeds, after ripening, attain light brown colour and emit an aromatic odour. It is distributed in Germany, Hungary, Netherlands, Pakistan and USA. In India, it is found in Rajasthan, Gujarat, J&K, Orissa, Madhya Pradesh and Punjab (Gupta et al., 2001). Dill seeds are used, both whole and ground, as a condiment in soups, salads, processed meats, sausages, spicy table sauces and in dill picklings. Grounded seed is an ingredient of seasoning. Dill stems and blossom heads are used for dill pickling and for flavouring soups. The essential oil is also used in the manufacture of soaps (Dahiya et al., 2012). Medicinally, essential oil of dill is considered to be carminative and specially used in control of flatulence, colic and hiccups in infants and children. In recent years the scientific literature reports pharmacological effects of dill such as antibacterial (Singh et al., 2001), antimycobacterial (Stavri et al., 2005), antioxidant (Lado et al., 2004, Ghasemi et al., 2009)..

2. Materials and Methods

2.1 Chemicals and reagents

Ascorbic acid, Gallic acid, Euginol, Toluene. Ethyle acetate, Formic acid, BHT (Butylated hydroxy toluene)

and 1-1-diphenyl-2-pic-rylhydrazyl (DPPH), were purchased from Sigma-Aldrich. All the solvents and chemicals (AR grade) are obtained from SD Fine Chemicals, Mumbai, India.

2.2 Physico-chemical analysis

Air dried leaves material was used for the quantitative determination of loss on drying, total ash, acid insoluble ash, alcohol, hexane and water soluble extractive values, according to standard procedure of Indian Pharmacopoeia and WHO/QCMMPM (API, 2003).

2.3 Preparation of plant extracts

The Plant material was manually screened for any impurities and dried in shade. For complete drying it was kept in hot air oven at 45˚C and then powered with an electric grinder. The coarse powder was subjected to methanolic extraction. Extracts were continuously stirred for 6 hrs and kept up to 18 hrs at room temperature. The process was repeated up to complete extraction. The extract was filtered and concentrated under vacuum in a rotatory evaporator (Buchi rotavpour, Switzerland) at 40 °C. The extract was finally freeze dried and stored at 4 °C for further use. 10 mg/ml of the extract was used for HPTLC studies.

2.4 *In- vitro* activities

2.4.1 *In-vitro* Antioxidant activity

Total flavonoid and phenolic content was estimated (Bray et al., 1954) and expressed in terms of mg/g of QE (Quercetin Equivalent) and mg/g GAE (Gallic Acid Equivalent) based on calibration curve of Quercetin and Gallic acid as standard. The anti oxidant potential was analyzed via DPPH radical scavenging assay (Liyana et al., 2005).

Total Antioxidant activity

This activity uses a reaction mixture containing concentrated 0.2M H_2SO_4, 28mM sodium phosphate and 4mM ammonium molybdate in distilled water. Three ml of this reaction mixture was added to 1ml of crude methanol extract prepared in different concentrations. After incubation at 95°C for 1hr in water bath absorbance was measured at 695nm using 3ml of reaction mixture and 1ml of distilled water as blank (Prieto et al., 1999).

Ferric reducing ability of plasma (FRAP) assay

Different concentrations of methanol extracts of the leaf was mixed with 2.5ml of phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide and incubated in water bath at 50°C for 20min. After incubation 2.5ml of 10% Trichloroacetic acid (TCA) is added. The entire mixture was centrifuged at 5000rpm for 10min and 2.5ml of the supernatant was added to 2.5ml milli Q water and 0.5ml of 0.1% ferric chloride ($FeCl_3$).It was again incubated at 50°C for 10 minutes and absorbance was measured at 700nm in UV-Spectrophotometer (Kumaran et al., 2007).

2.4.2 *In-vitro* Antidiabetic activity

DNS Method (3, 5-Dinitrosalicylic acid method)

The inhibition assay was performed using standard DNS method (Millar et al., 1959). The results were expressed as % inhibition calculated using the following formula-

Inhibition activity (%) = Abs (Control)-Abs (extract) × 100 / Abs (Control)

Starch-iodine color assay

Assay was carried out with slight modification based on the starch-iodine test (Xiao et al., 2006). Inhibition of enzyme activity was calculated as:

Inhibition of enzyme activity (%) = (C-S) / C × 100

where S is the absorbance of the sample and C is the absorbance of blank (no extract).

2.5 High Performance Thin Layer Chromatography

2.5.1 Preparation of plant extract

The coarsely powdered drug was completely extracted in methanol. The extract was filtered, dried and a stock solution of 10 mg/ml was prepared. The solution was filtered through a 0.45 µm Millipore membrane filter (Pall, USA). Now, a working solution of 1.0 mg/ml was prepared for HPTLC studies.

2.5.2 Preparation of standard compound solution

The stock solution of Lupeol (1 mg/ml) was freshly prepared in methanol and was stored in the dark. Four concentrations of the working solution were prepared by taking 0.1, 0.2, 0.3, 0.4 ml from the stock solution respectively in separate vials and making it up to 1 ml with methanol. The solutions were filtered through a 0.45 µm Millipore membrane filter (Pall, USA) before application.

2.5.3 HPTLC conditions

High Performance Thin Layer Chromatography is a sophisticated and automated form of TLC technique. This method is used for separation of the components present in mixture, both quantitatively as well qualitatively. For quantitative analysis of medicinal

plant sufficient quantity of ethanol extract after dissociating in methanol was passed through Millipore membrane filter unit. About 8µl was applied on pre-coated plates with silica gel $60F_{254}$ of 0.2 mm thickness as 6 mm-wide bands positioned 10 mm from the bottom and 15 mm from side of the plate, using CAMAG LINOMAT V automated TLC applicator with nitrogen flow providing a delivery speed of 150 ml/s from application syringe. Following sample application, layers were developed in a CAMAG twin trough glass chamber which was pre-saturated with mobile phase Hexane: Ethyl acetate: Acetic acid (8:4:2 v/v). After development of the plate, it was dried and then scanned at 400nm with a TLC scanner (WINCATS 1.3.2, CAMAG).

Calibration curve

Apply 2,4,6 µl of standard solution in duplicates on the TLC plates developed the solvent system to a distance of 8 cm. Dried the plate to obtained the chromatogram and determined the area of peak corresponding to that of lupeol as described given bellow for the calibration curve by plotting peak area Vs concentration of lupeol.

3. Results and Discussion

Natural antioxidants are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. In the present paper, we have evaluated the free radical scavenger activity of methanolic extract of *Anethum sowa*. The aim of our study is to provide scientific evidence concerned to the medicinal values of these unexplored plants. Air dried seed material was used for the determination of loss on drying, total ash, acid insoluble ash, water insoluble ash, water soluble and alcohol soluble extractive value were shown in Fig-1.

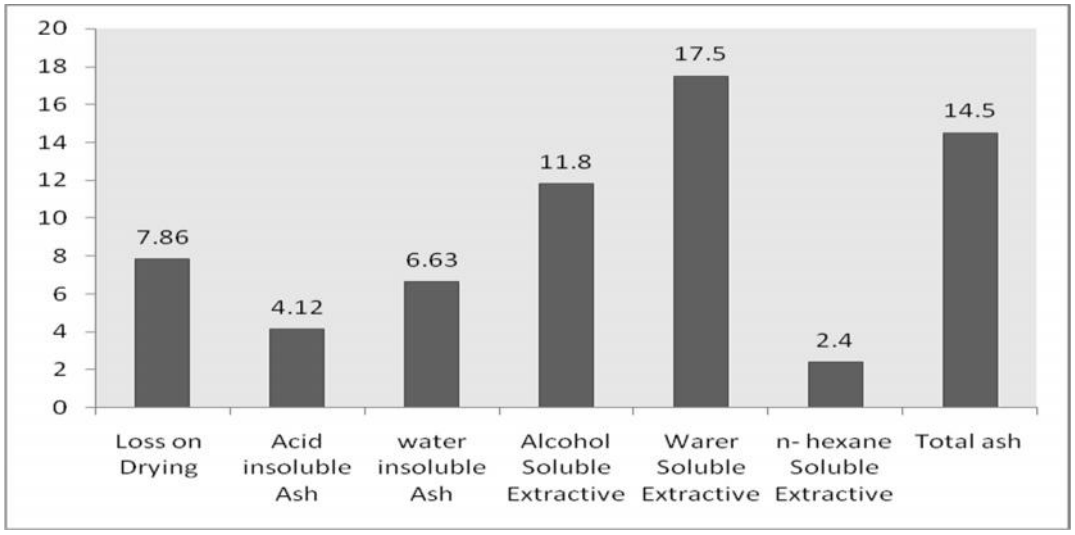

Figure: 1. Physico-chemical parameters of *Anethum sowa*

The antioxidant potential of the plant was further determined by the total antioxidant capacity, total reducing, and DPPH scavenging assay were shown in Fig-2, Fig-3 and Fig-4 respectively. The total antioxidant capacity of *Anethum sowa* extract was measured by phosphor-molybdenum method based on regression analysis of ascorbic acid, having regression equation ($y = 0.001x + 0.0552$) and coefficient; $r^2 = 0.9852$. Reducing power of the species increases linearly with concentration, similar to standards.

Free radical scavenging activity of DPPH is most widely used for screening of medicinal plants having anti oxidant activity. The mechanism however, well evident is due to de-colorization of DPPH through electron donated by anti oxidant compound/moiety and thus stabilizing the DPPH radical. The IC_{50} value for the *in vitro* DPPH method was 1.22 ± 0.411 mg/ml.

Diabetes mellitus is an endocrine disease that is developing along with an increase in both obesity and ageing in the general population. The treatment aim of diabetic patients is to maintain near normal levels of glycemic control, in both the fasting and postprandial states. In particular, α-amylase participates in glucose digestion and is considered as key enzymes that can control postprandial hyperglycemia. In present study, it was found that the plant extract showed significant antidiabetic activity. Antidiabetic activity was observed by 3.5 DNS (Fig-5) and starch iodine model (Fig-6), was found to be most potent antidiabetic. In the starch-Iodine assay IC_{50} of methanolic extract was 252.9 ± 0.05 µg/ml and 0.236 ± 0.04 mg/ml for the DNS method.

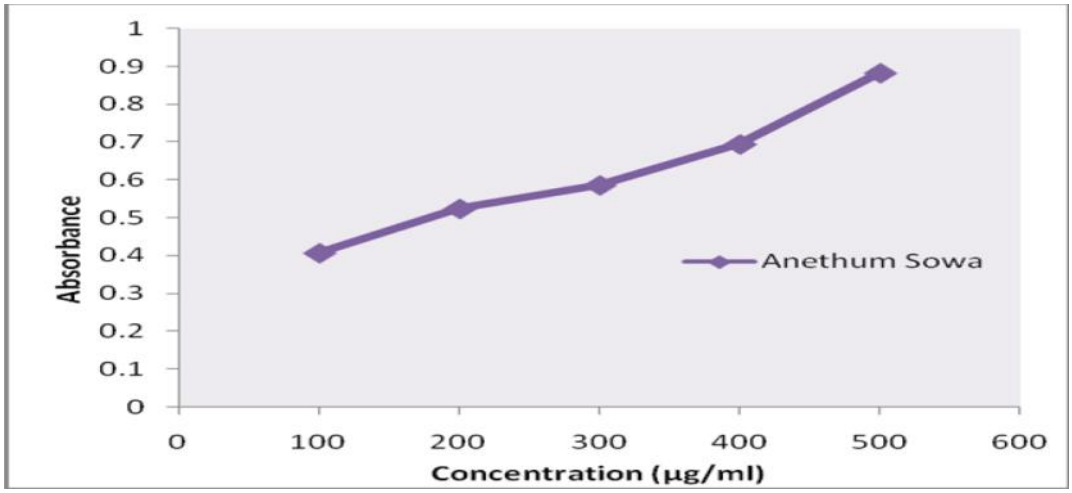

Figure: 2. Total anti oxidant capacity of *Anethum sowa*

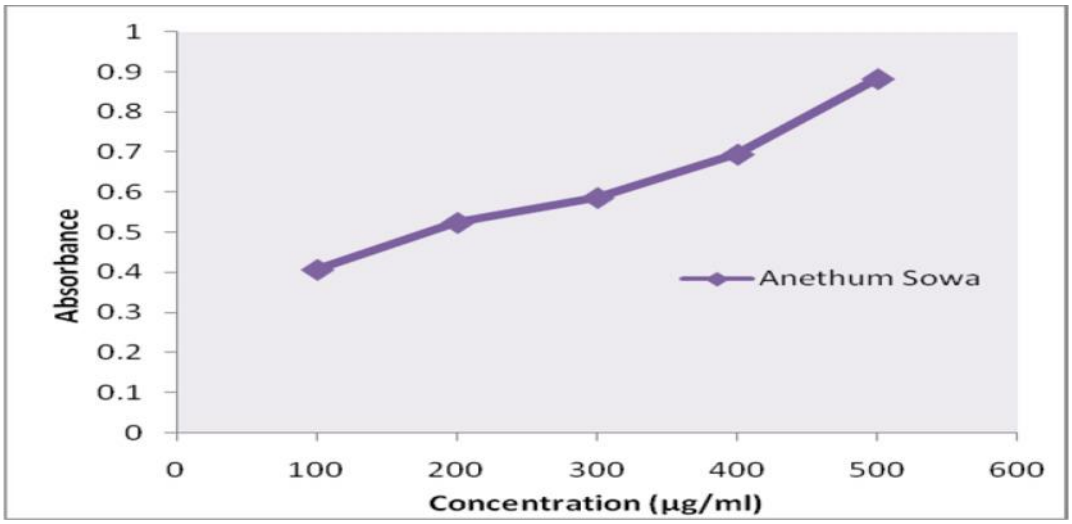

Figure: 2. Total anti oxidant capacity of *Anethum sowa*

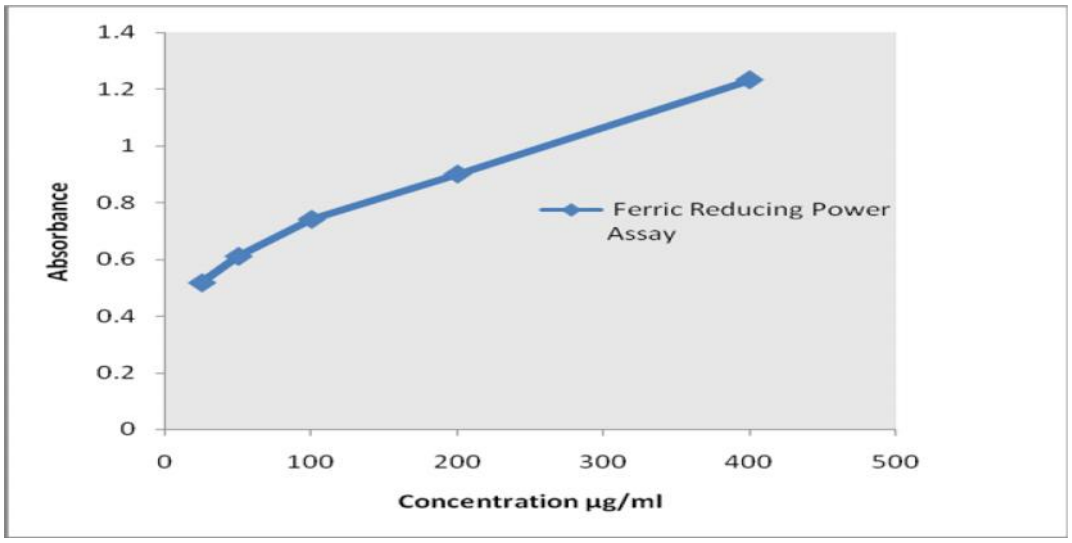

Figure: 3. Ferric Reducing assay of *Anethum sowa*

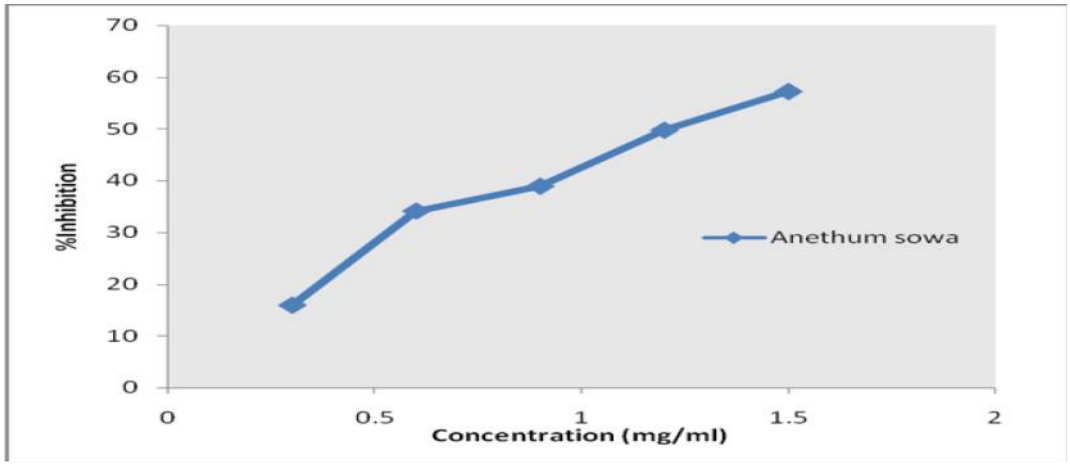

Figure: 4. DPPH radical scavenging activity in methanolic extract of *Anethum sowa* Linn. (Values are mean ± S.D).

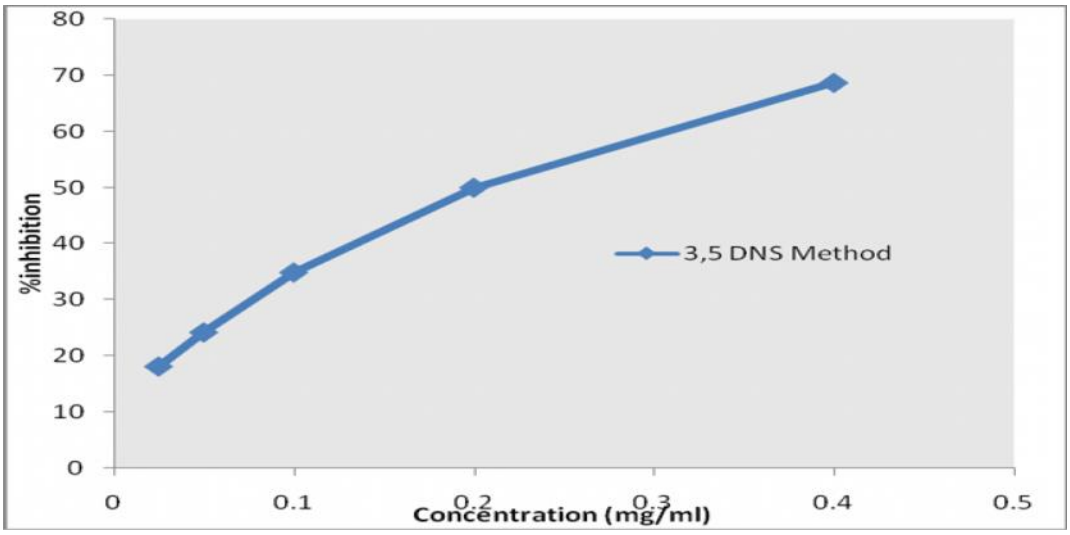

Figure: 5. 3, 5-dinitrosalicylic acid assay of *Anethum sowa*

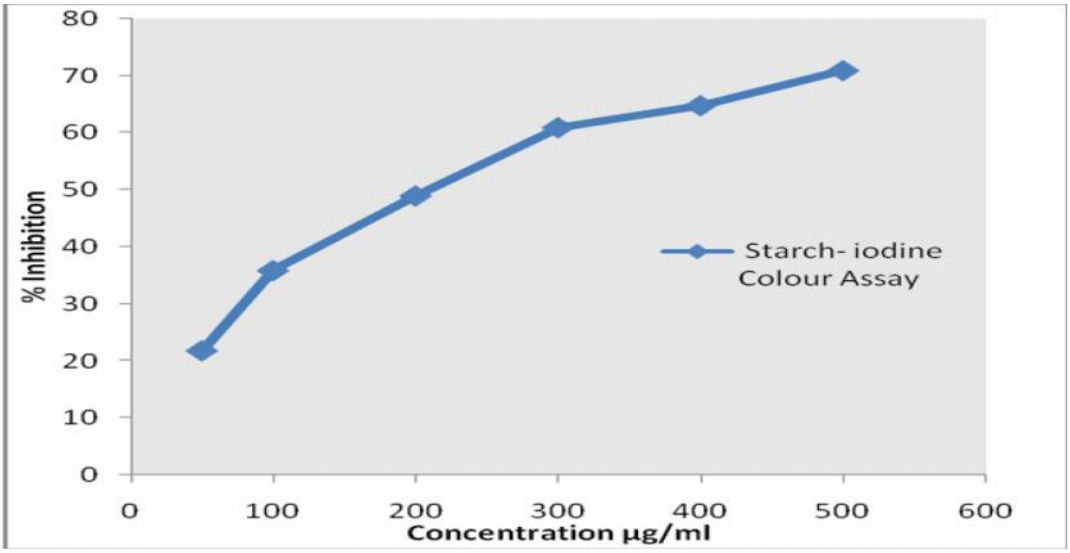

Figure: 6. Starch-iodine color Assay of *Anethum sowa*

Quantification of Lupeol by HPTLC in *Anethum sowa* was carried out on the basis of calibration curve of standard, linearity graph and track of lupeoll were shown in Fig-7, Fig-8 and Fig-9. Three dilutions of standard were used in concentration range of 2, 4 and 6 µg/ml and various calibration values were obtained. Concentration of Lupeol was found in *Anethum sowa* is 0.0018mg/ml.

Figure: 7. HPTLC Plate of *Anethum sowa*

Abrebiation :- L_1, L_2, L_3 - Lupeol , AS- *Anethum sowa*

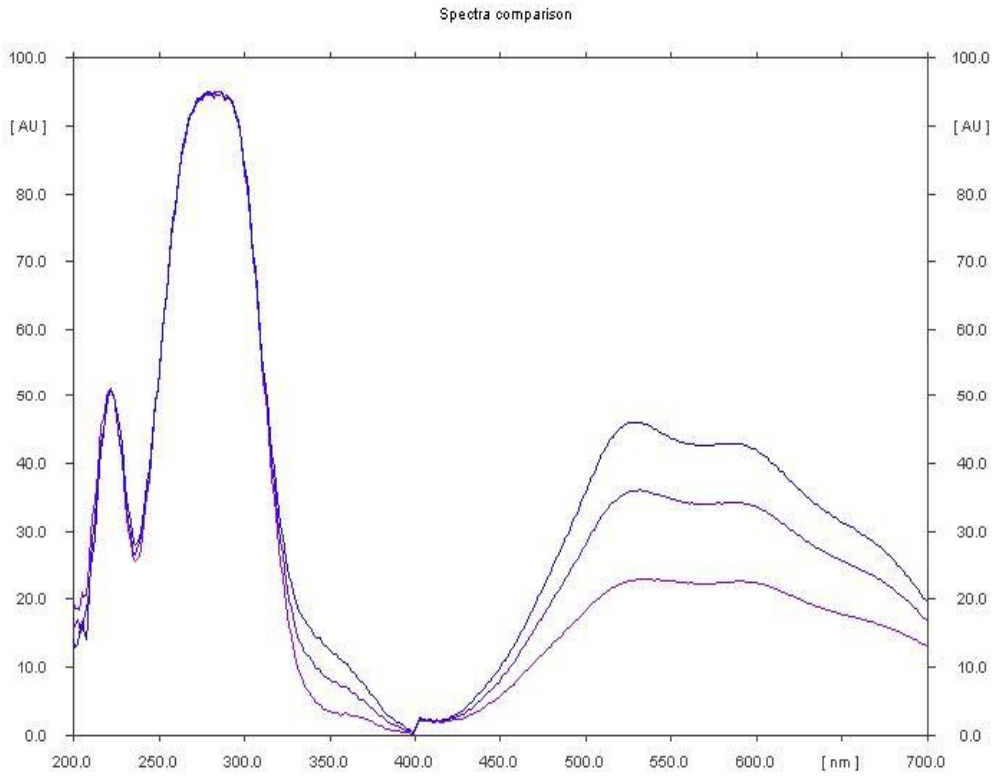

Figure: 8. Linearity

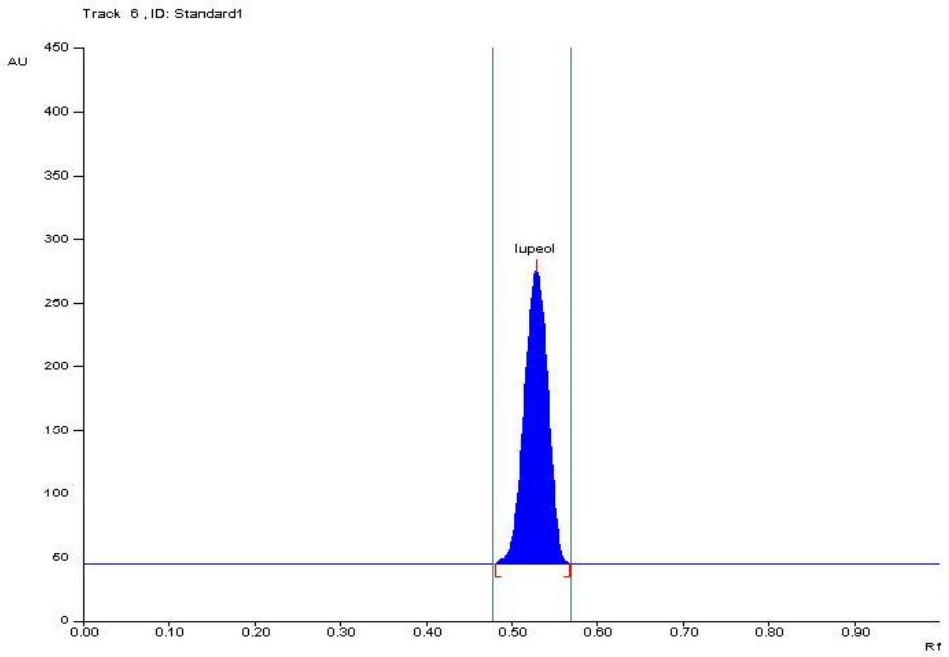

Figure: 9. Track of Lupeoll

Conclusion

The results obtained from loss on drying, total ash, acid insoluble ash, water soluble and alcohol soluble extractive value will be helpful in the identification, standardization and quality control of the drug. In the present study antidiabetic and antioxidant activities of methanol extract of *Anethum sowa* were investigated and plant shows considerable amount of the activities. Among antidiabetic studies, enzyme inhibitory activities (α amylase inhibitory assay) and in case of antioxidant studies, DPPH radical scavenging assay, reducing power assay, total antioxidant activity estimation were conducted. The free radicals generated due to oxidative stress can be seen as a major cause for diabetes. Since *Anethum sowa* has antidiabetic and antioxidant activity, it can be as a major cure for diseases resulting from free radicals.

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