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ISOLATION, PHYTOCHEMICAL INVESTIGATION AND BIOLOGICAL SCREENING OF LEAF OF CHROZOPHORA ROTTLERI

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ABSTRACT

Phytochemical screening of Euphorbiaceae members were carried out for understanding their solvent solubility. Qualitative analysis of secondary metabolites was performed for the presence of Alkaloids, Tannins, Proteins, Flavanoids, Phenols, Steroids, Saponins, Quinines, Glycosides, Carbohydrates and Amino acid. The results revealed that in all studied species (Chrozophora rottleri) shows negative response for steroids test indicates usefulness of these plants as food and fodder. The results also revealed that Euphorbiaceae members have maximum phytochemicals which are soluble in organic solvent. Chrozophora rottleri is a herb plant, which is found in Africa, India, Pakistan, Australia, Myanmar, and Central Asia. Chrozophora plicata in Indian vegetations is known as Chrozophora rottleri. The plant has a spot with the Euphorbiaceae family. The plant is erect with Silvery hairs, stem, and leaf parts are uncovered and bristly. The season for starting to develop is February to August. It appears in the month of February, and flowering in June and July and it completely disappears in the late August. The plant frequently grows at the edges of the paddy field; plant shows luxuriant growth in moderate level of watering. The plant has an extremely huge number of chemical constituents and different pharmacological properties. The plant contains chemical synthetics like Alkaloids, sugar, glycosides, tannins, steroids, flavonoids etc., Leaf and basic pieces of C. rottleri were rich in xanthone glycosides and chromone glycoside. Oil dispensed from seeds was rich in lineolate and the whole plant contains tannins. Leaves and root powder is given in the treatment of cold, cough, and wound recuperating. The plant Chrozophora rottleri shows antimicrobial, anti-oxidant, ant necrotic, antihelmintic properties.

Keywords: Euphorbiaceae, Chrozophora Rottleri, Glycosides, Anti Inflammatory, Antimicrobial, Antioxidant.

I. INTRODUCTION

Diabetes mellitus (DM), a metabolic issue around the world, is described by hyperglycemia connected with weakness in insulin secretion and adjustment in digestion system of starch and lipids. Diabetes mellitus is the third driving reason for death in numerous nations after cardiovascular diseases and cancer. The greater parts of plants discovered contain substances like glycosides, alkaloids, flavanoids, saponins etc.,The plant Chrozophora rottleri contains this type of chemical constituents and the presence study was undertaken, this paper provides detail and distinctive anti-diabetic analysis of the plant. Extraction is process where separation of medicinally active compounds from plant tissues using selective solvents through standard procedures. In Sudan, stem powder is utilized in mending of wounds and treatment of Jaundice in India, In Nepal the leafy foods of the plant utilized in treatment of cold and cough. Leaf powder is utilized in the treatment of Skin like sun burn and sunstroke. Leaves are used to treat Leucoderma. It is utilized in Ayurvedic like Emetic, Cathartic, Purgatives, and Depurative. Concentrate of leaf has hostile to helmintic property against Pheritima posthuma (Indian Earth worm) and phytotoxic action on Rice, wheat and mustard. Seeds are utilized as Laxatives and Purgatives. The Extraction of plant with methanol shows the greatest inhibition in Gram positive and Gram negative microbes[1,2].

TAXANOMICAL CLASSIFICATION

Kingdom	Plantae
Order	Malpighilales.
Family	Euphorbiaceae.
Subfamily	Acalyphoideae.
Tribe	Chrozophoreae.

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Subtribe	Chrozophorinae.
Genus	Chrozophora Neck.
Species	Rottleri

Preparation of plant material:

Procedure:

The leaves of these plants were washed thoroughly under running tap water and then dry at Room Temperature for 2 hours and then dried in an oven at 60°C for 8 hours. The dried plant material were pulverized to fine powder in a grinder, stored in air tight bottle, labeled and kept in a dark room[3].

Fig 1: Preparation of plant leaf material

Maceration:

Extraction of leaves of respective plants was carried out by maceration technique. The solubility of sample checked with different solvents, the leaf powder was freely soluble in the ethanol, methanol and ethyl acetate. 2 gm of dried powder was macerated separately in 20 ml of ethanol, methanol and ethyl acetate in test tube. The flasks were covered with aluminum foil and allowed to stand in a dark for 72 hrs for extraction. These extracts were filtered and the filtrate was evaporated to dryness in heating plate[4].

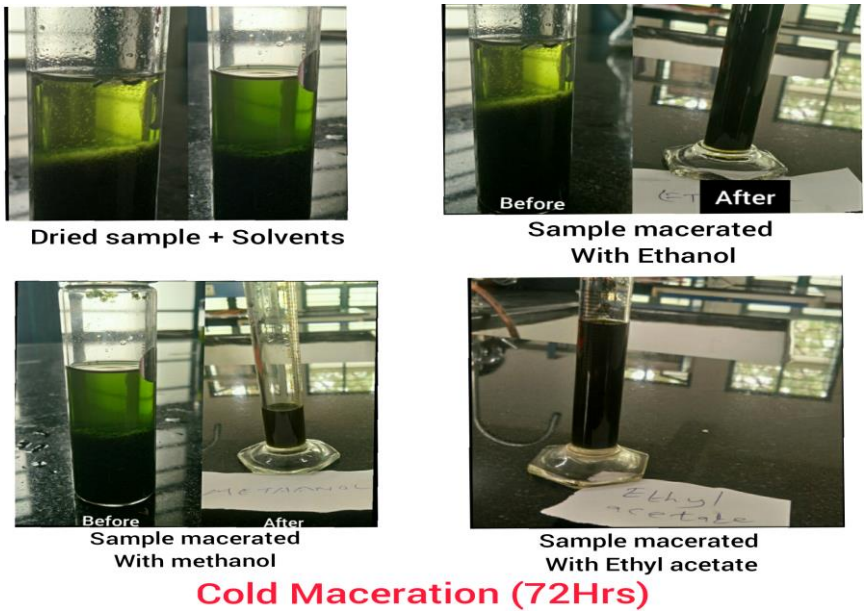

Fig 2: Maceration of the leaf powder

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Concentration:

The Methanol, Ethanol and Ethyl acetate extract collected in the china dish and kept for the concentration at the temperature of 50^0C.

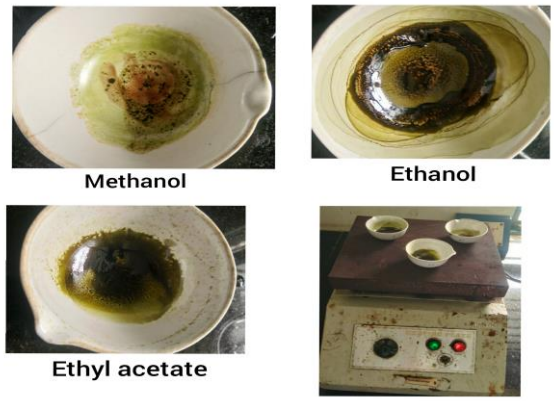

Fig 3: Concentration of macerated product

Chromatography:

Thin layer chromatography:

Two grams of powdered sample was macerated with 20 ml consecutively with three solvents such as methanol, ethanol and ethyl acetate then concentrated and dried. The final extract was re-dissolved with non-polar solvents such as Toluene, Ethyl acetate. It is used for the TLC analysis. Pre-coated Silica Gel plate was used for stationary phase and three types of mobile phase is used for the separation such as Toluene: Ethyl acetate (9.0:1.0), Hexane: Ethyl acetate (9.3:0.7), Toluene: Hexane (9.5:0.5) undergoes separation. After development the plate was dipped in Iodine chamber for 2 minutes to develop the color and the spots were recorded[5].

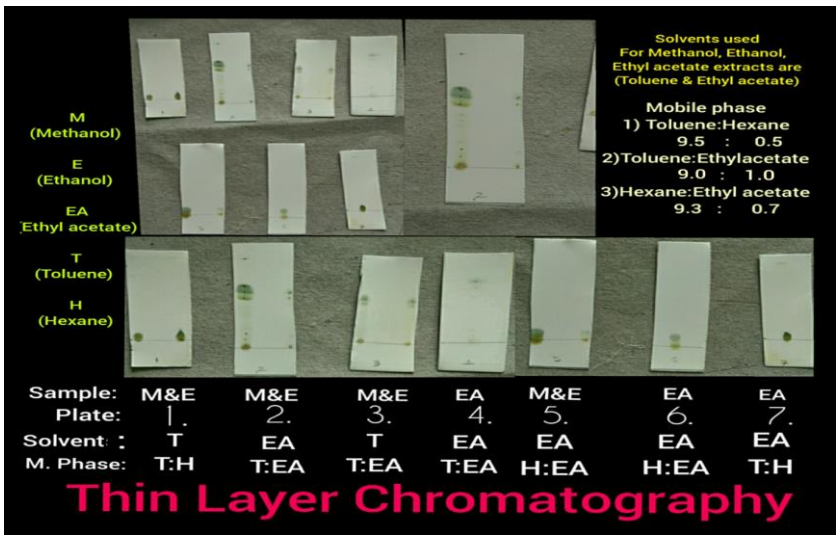

Fig 4: TLC preparation of macerated samples

Plate 1: Methanol and Ethanol extract dissolve in Toluene and undergoes separation with the mobile phase (Toluene: Hexane).

Plate 2: Methanol and Ethanol extract dissolve in Ethyl acetate and undergoes separation with the mobile phase (Toluene: Ethyl acetate).

Plate 3: Methanol and Ethanol extract dissolve in Toluene undergoes separation with mobile phase (Toluene: Ethyl acetate).

Plate 4: Ethyl acetate extract dissolve in Ethyl acetate undergoes separation with mobile phase (Toluene: Ethyl acetate).

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Plate 5: Methanol and Ethanol extract dissolve in Ethyl acetate undergoes separation with mobile phase (Hexane: Ethyl acetate).

Plate 6: Ethyl acetate extract dissolve in Ethyl acetate undergoes separation with mobile phase (Hexane: Ethyl acetate).

Plate 7: Ethyl acetate extract dissolve in Ethyl acetate undergoes separation with mobile phase (Toluene: Hexane).

The Plates 2, 3 and 4 has better separation with the mobile phase (Toluene: Ethyl acetate) comparing to other mobile phases and methanol was freely soluble with many chemical constituents.

Dyes separation Chart:

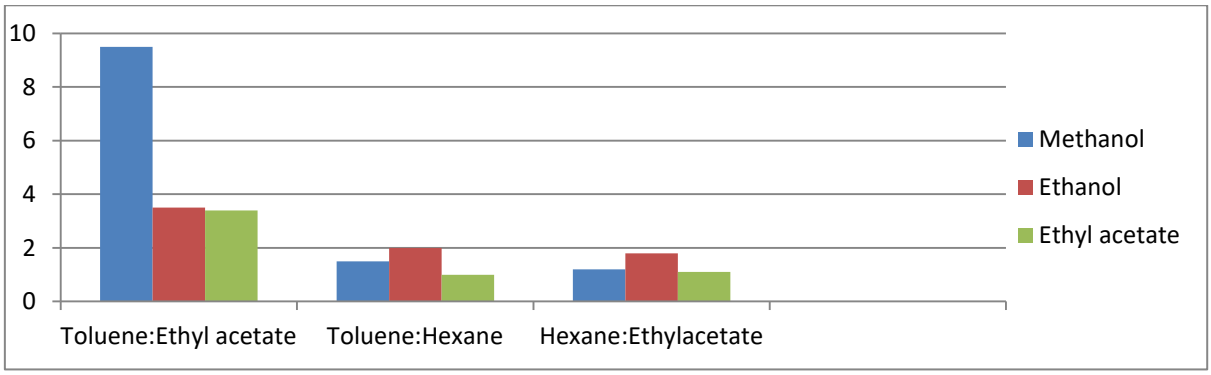

Fig 5: TLC separation chart

R_f (Retardation Factor) value:

$$R_f = \frac{\text{Distance travelled by the substance.}}{\text{Distance travelled by the solvent front.}}$$

Plate 2:

Methanol Extract:

The distance travelled by solvent = 4.7cm.

Distance travelled by the various dyes(10) = 3.3cm, 3cm, 2.7cm, 2.2cm, 2cm, 1.7cm, 1.3cm, 1cm, 0.8cm, 0.5cm.

(R_f value = 0.71, 0.64, 0.58, 0.47, 0.43, 0.37, 0.26, 0.22, 0.18, 0.11).

Ethanol Extract:

The distance travelled by solvent = 4.7cm.

Distance travelled by the various dyes (2) = 2.9cm, 2.6cm.

(R_f value = 0.62, 0.56).

Plate 3:

Methanol Extract:

The distance travelled by solvent = 4.6cm.

Distance travelled by various dyes (3) = 2.3cm, 2cm, 1.5cm.

(R_f value = 0.51, 0.44, 0.33).

Ethanol Extract:

The distance travelled by solvent = 4.6cm.

Distance travelled by various dyes (2) = 2.4cm, 2.1cm.

(R_f value = 0.53, 0.46).

Plate 4:

Ethyl acetate Extract:

The distance travelled by solvent = 4.8cm.

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Distance travelled by various dyes (2) = 3.4cm, 3cm.

(R_f value = 0.71, 0.63).

Soxhlet Extraction:

Fig 6: Soxhlet apparatus for extracion and concentrating extracted product

1. 20 grams of sample placed in the porous thimble of the siphon tube. The methanol was used as solvent for the extraction.
2. The solvent was heated to reflux. The solvent vapor travels up a distillation arm and floods into the chamber of thimble containing solid sample.
3. The desired compound will then dissolve in the warm solvent, when the soxhelt chamber is almost full; the chamber is automatically emptied by siphon side arm, with the solvent running back to the distillation flask.
4. The extracted solvent allowed for concentration and undergoes for the further process of separation under chromatography[6].

Column Chromatography:

Fig 7: Separation of Bands using Column Chromatography

Procedure:

1. After the Extraction, the column was used for the isolation of the compound.
2. The mobile phase was followed based on the TLC. The solvent used for the isolation of the different fraction are Toluene: Ethyl acetate in the ratio (9:1).
3. The column was filled with the Silica gel using the mobile phase. (1gm of silica gel in 10ml of mobile phase.) The process kept for saturation for two hours for activation of silica gel.
4. The column was run in the velocity of one drop in nine seconds in order for the proper separation of fraction. Nine bands were obtained are collected for the further process[7].

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Phytochemical test:

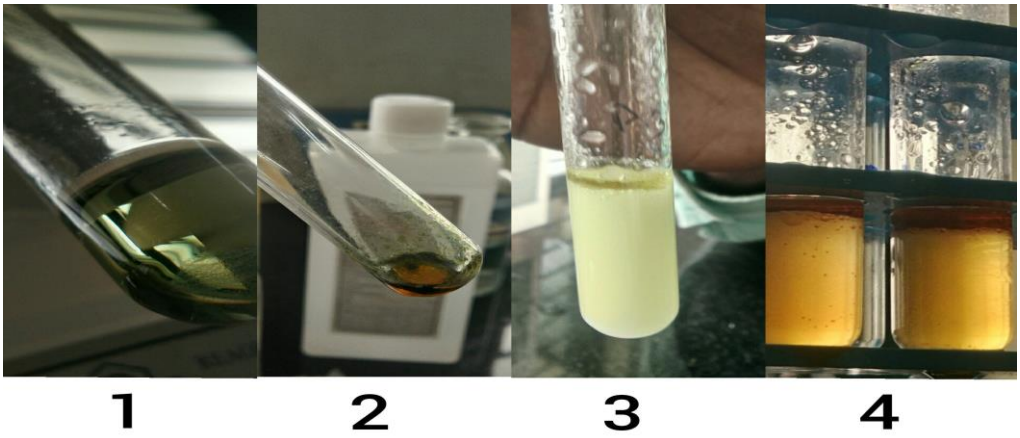

Figure 8: 1) Quinine Test, 2) Alkaloid test, 3) Saponin test,4) Carbohydrates test.

Phytochemicals	Test	Observation	Inference
Quinine(**1**)	Herpathite test	Crystal formation	+
Alkaloids(**2**)	Wagners test	Reddish Brown ppt.	+
Saponin(**3**)	Foam test	Presence of emulsion	+
Carbohydrates(**4**)	Benedicts test	Two layer formation	+
Glycosides	Lieberman test	_	_
Flavanoids	Shinoda test	_	_

Preliminary phytochemical analysis:

IR Spectroscopy:

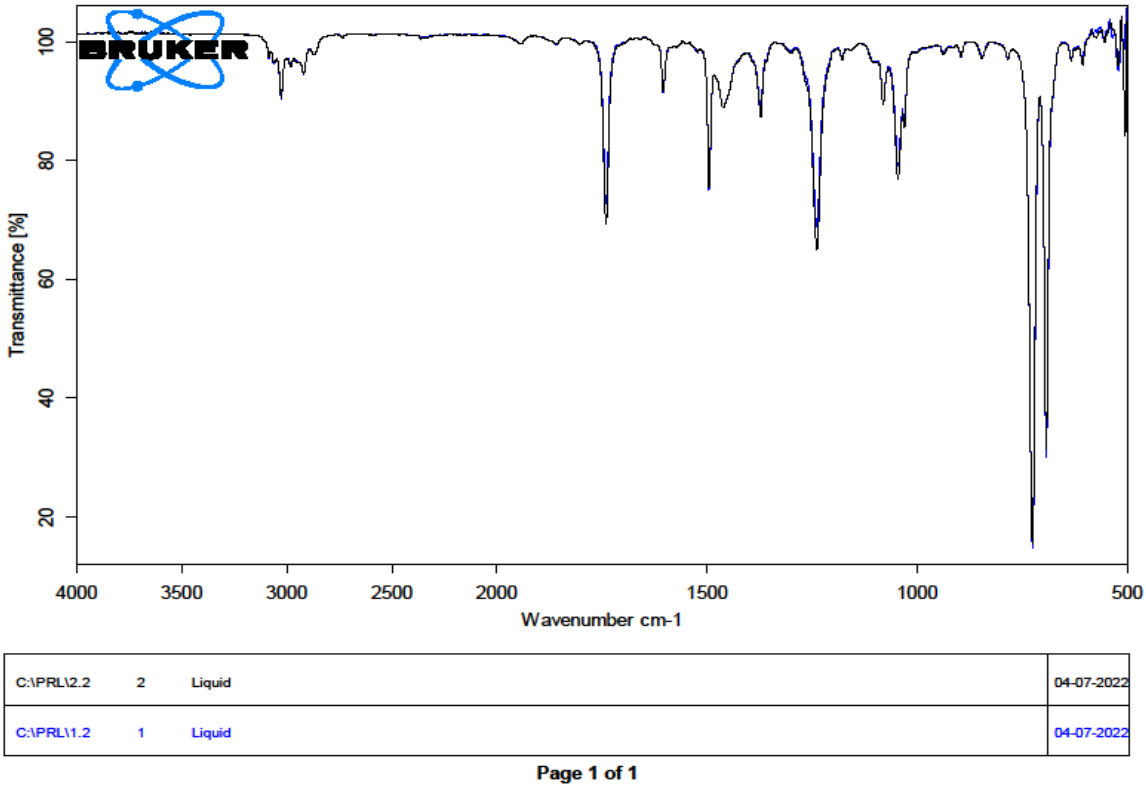

Fig 9: IR Spectrum of Chrozophora rottleri plant extract

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1. Qualitative analysis of methanolic extracts of Chrozophora rottleri was performed for the identification of various classes of active chemical constituents like alkaloids, carbohydrates, glycosides, proteins, amino acids, steroids etc.

2. IR Spectroscopy is used to establish whether a given sample of an organic substance is identical with another or not. This is because large number of absorption bands is observed in the IR spectra of organic molecules and the probability that any two compounds if produce identical spectra is almost zero. So, if two compounds have identical IR spectra then both of them must be samples of the same substances.

LC-MS Analysis:

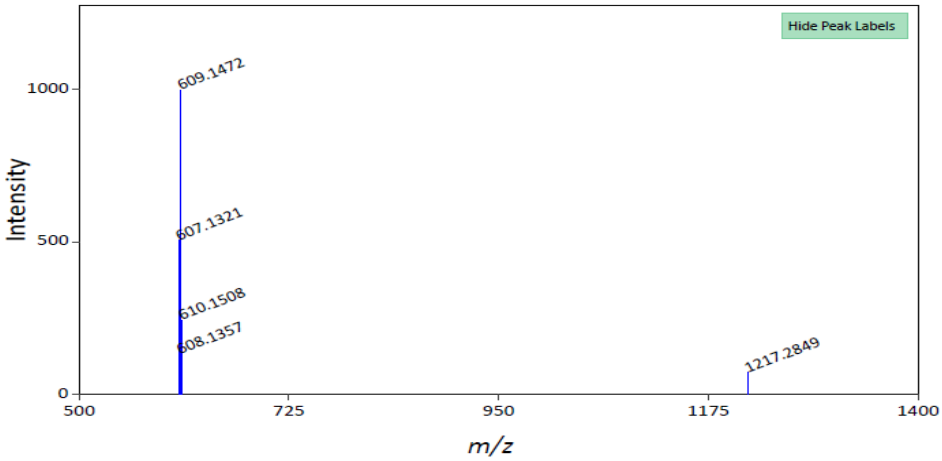
Chromatographic estimation of Flavanoids in Methanol extracts of Chrozophora rottleri. It is routinely used in phytochemistry for analytical as well as preparative separation purposes. For chemo-taxanomic purposes, the botanical relationships between different species can be shown by chromatographic comparison of their chemical composition. Comparison of chromatograms, used as fingerprints, between authentic samples and unknowns permits identification of drugs and/or search for adulteration. LC-MS is thus, very effective technique for an efficient separation of crude plant extracts. Analysis of components present in methanol extracts of Chrozophora rottleri was done by selecting a standard compound. Flavanoid Glycosides was found to be one of an active compound in Chrozophora rottleri. Studies reported that Apigenin (Flavanoids) exerts anti-diabetic properties by suppressing adipogenesis in 3T3-L1 cells by activating 51AMP-activated protein kinase (AMPK) and by inhibiting mitotic clonal expansion. LC-MS analysis of methanol extracts of Chrozophora rottleri was made by identifying the Apigenin derivatives present in the sample.

Active Constituents:

The investigation revealed that the aerial parts of this plant contain flavone, methylated flavones, glycosides and acylated glycosides. C. rottleri was found to contain apigenin, apigenin 7-O-methyl ether, apigenin 7-O-β-D glucopyranoside, apigenin 7-O- (6""-E-pcoumaroyl)- β -D- glucopyranoside (a rare flavonoid) and apigenin 7-O-(3""-E-pcoumaroyl)-β -D- glucopyranoside (a new acylated flavonoid). The occurrence of flavanones is the first report from the species Chrozophora rottleri. The flavones apigenin is found to be very common in the species of Chrozophora, especially in C.senegalensis, C. tinctoria, C. brorcchiana, C. rottleri and C. plicata[8].

1. quercetin-3-o-rutinoside(1-Rutin) (Fig.10).
2. 5,7,4`-trihydroxyflavone(apigenin) (Fig.11).
3. Apigenin 7-o-beta-D-glucopyranoside (Fig.12).
4. acacetin-7-o-rutinoside(Linarin) (Fig.13).

Quercetin-3-o-rutinoside (Fig.10):



Retention Time	295.2 sec	
Top 5 Peaks	609.1472 999, 607.1321 506, 610.1508 244, 608.1357 131,	
	1217.2849 71.	

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Molecular formula = $C_{27}H_{30}O_{16}$, Molecular weight=610.5g/mol, Melting point = 125^{0}C.

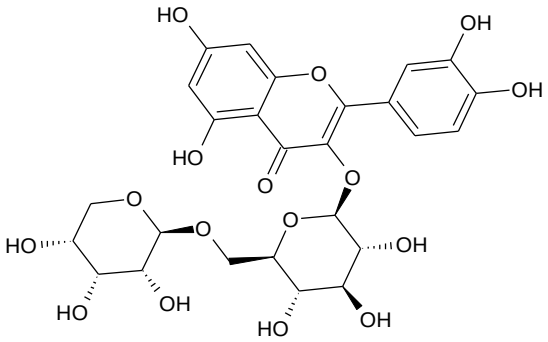
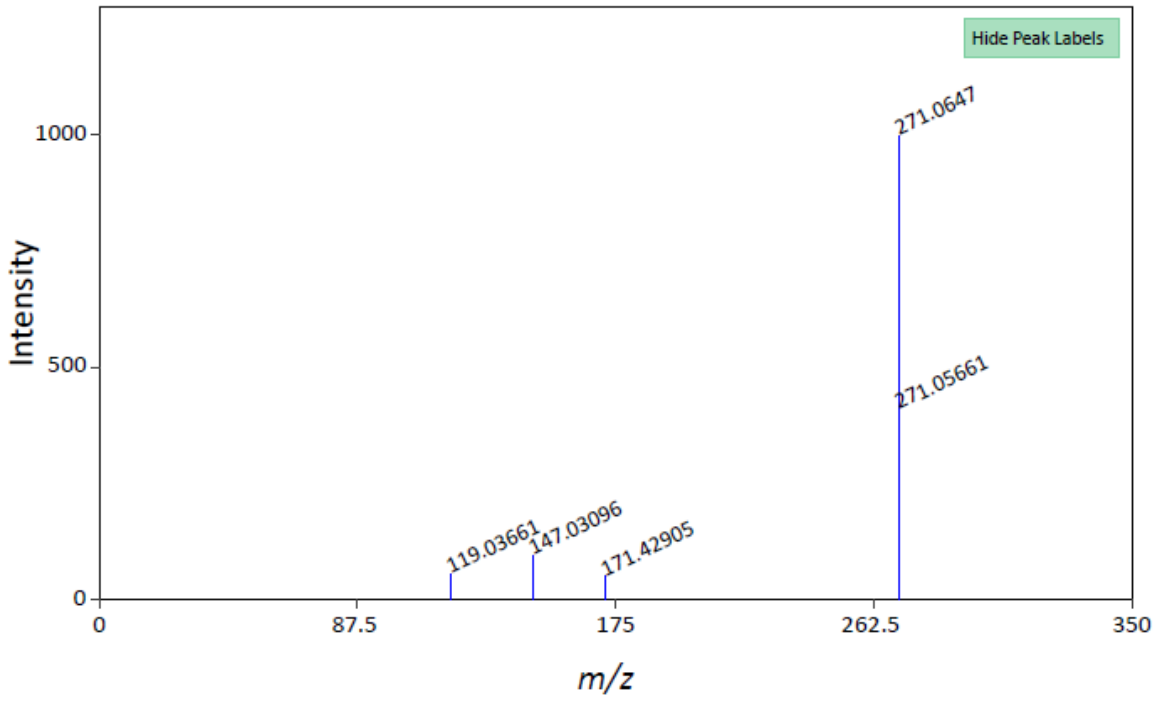


Fig 10: Quercetin-3-o-rutinoside(1-Rutin)

Uses: Rutin, quercetin-3-O-rutinoside, is a flavonoid that is widely distributed in plants and their products; it showed a significant antidiabetic effect via improvement of glucose uptake in vitro and in vivo, increasing insulin release, decreasing the level of blood glucose in STZ-treated rats as well as inhibition of α-glucosidase activity.

5,7,4`-trihydroxyflavanone(Fig.11):



Retention Time	4.16
Top 5 Peaks	271.0647 999, 271.05661 409, 147.03096 95, 119.03661 56,
	171.42905 51.

Molecular formula = $C_{15}H_{10}O_5$, Molecular weight=272, Melting point = 348- 350°C.

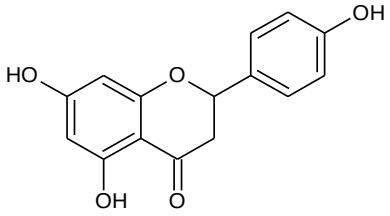


Fig 11: 5,7,4`-trihydroxyflavanone(apigenin)

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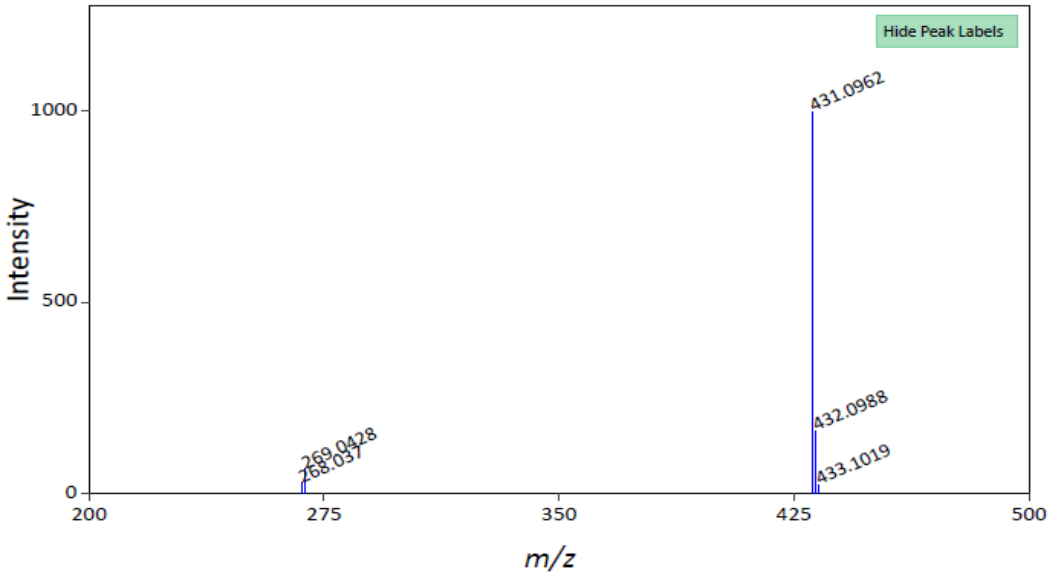
Uses: It is a natural flavonoid, possesses a broad spectrum of biological properties, including anti-oxidative, antidiabetic, anti-inflammatory, anticancer, and neuro-protective effects.

Apigenin 7-O-Beta-D-glucopyranoside(Fig.12):



Retention Time	388.2 sec
Top 5 Peaks	431.0962 999, 432.0988 164, 269.0428 65, 268.037 29,
	433.1019 23.

Molecular formula=$C_{21}H_{20}O_{10}$, Molecular weight=432.4g/mol, Melting point= 788.93°C.

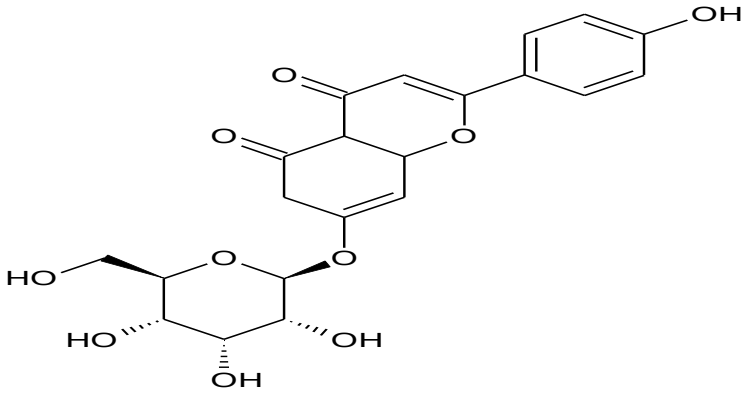


Fig 12: Apigenin 7-O-Beta-D-glucopyranoside.

Uses: Apigenin-7-glucoside (Apigenin-7-O-β-D-glucopyranoside) exhibits significant anti-proliferative and antioxidant activity and scavenges reactive oxygen species (ROS). Apigenin 7-glucoside exhibits significant anti-proliferative activity against B16F10 melanoma cells.

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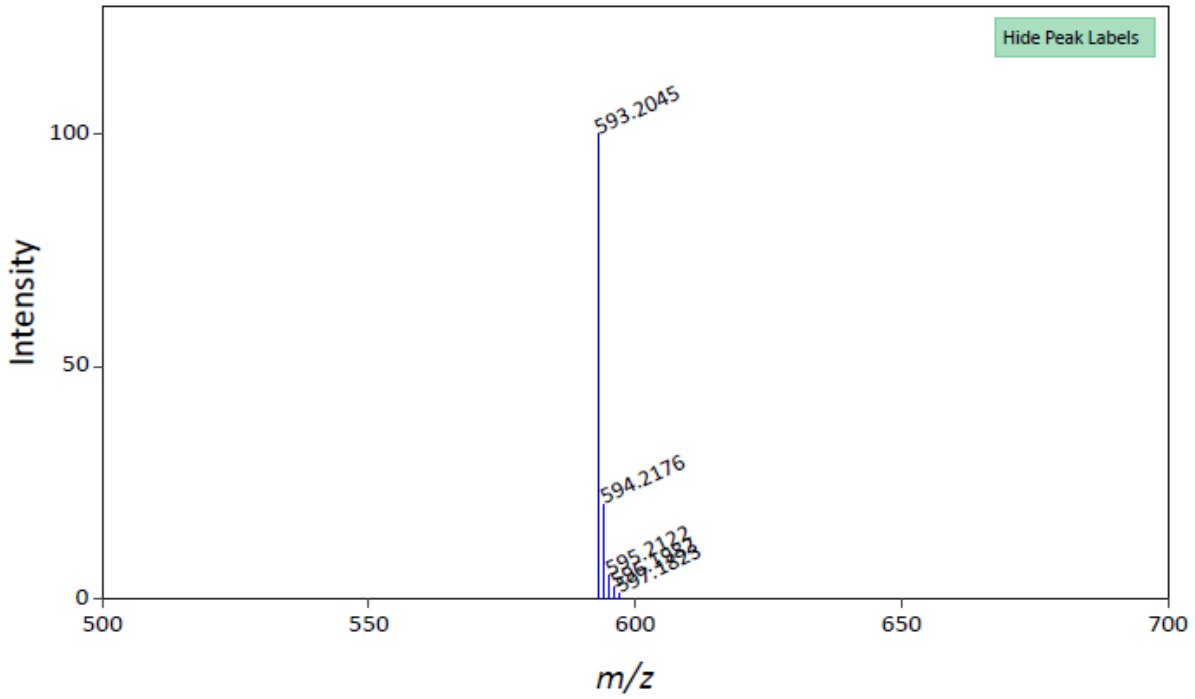
Acacetin-7-o-rutinoside(Fig.13):



Retention Time	21.39 min
Top 5 Peaks	593.2045 100, 594.2176 20.43, 595.2122 5.04, 596.1982 2.51, 597.1823 1.14.

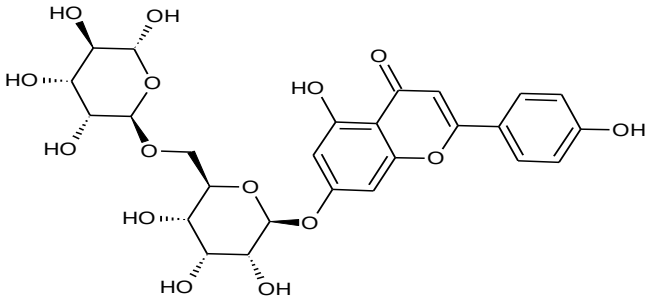


Fig 13: acacetin-7-o-rutinoside(Linarin)

Molecular formula = $C_{28}H_{32}O_{14}$, Molecular weight= 592.5g/mol, Melting point = 267–269°C.

II. RESULTS AND DISCUSSION

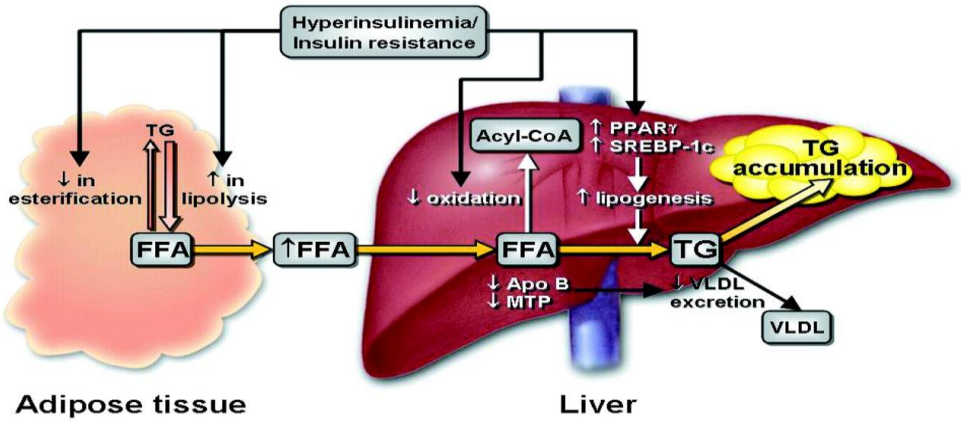


Fig 14: Liver antidiabetic activity

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Apigenin said to be anti-diabetic agent as it can suppress the activity of α-glucosidase, stimulate insulin secretion, and manage reactive oxygen species, which can manage diabetic complications. Apigenin can provide the endothelial cells by nitric oxide, and hence, prevent or decrees damage of endothelial cell that result from increase glucose level in blood. Apigenin decreased the activities of hepatic enzymes controlling triglyceride synthesis and cholesterol esterification and increased the expression of hepatic genes involved in fatty acid oxidation, the TCA cycle, OXPHOS, the electron transport chain and cholesterol homeostasis while decreasing the expression of hepatic lipogenic and lipolytic genes, indicating that these changes may be potential mechanisms for improving dyslipidemia and hepatic steatosis in HFD-fed mice. Moreover, apigenin decreased plasma pro-inflammatory adipocytokines levels and hepatic gluconeogenic enzyme activities, which may be partly associated with the improved hyperglycemia, hyperinsulinemia and insulin resistance[9].

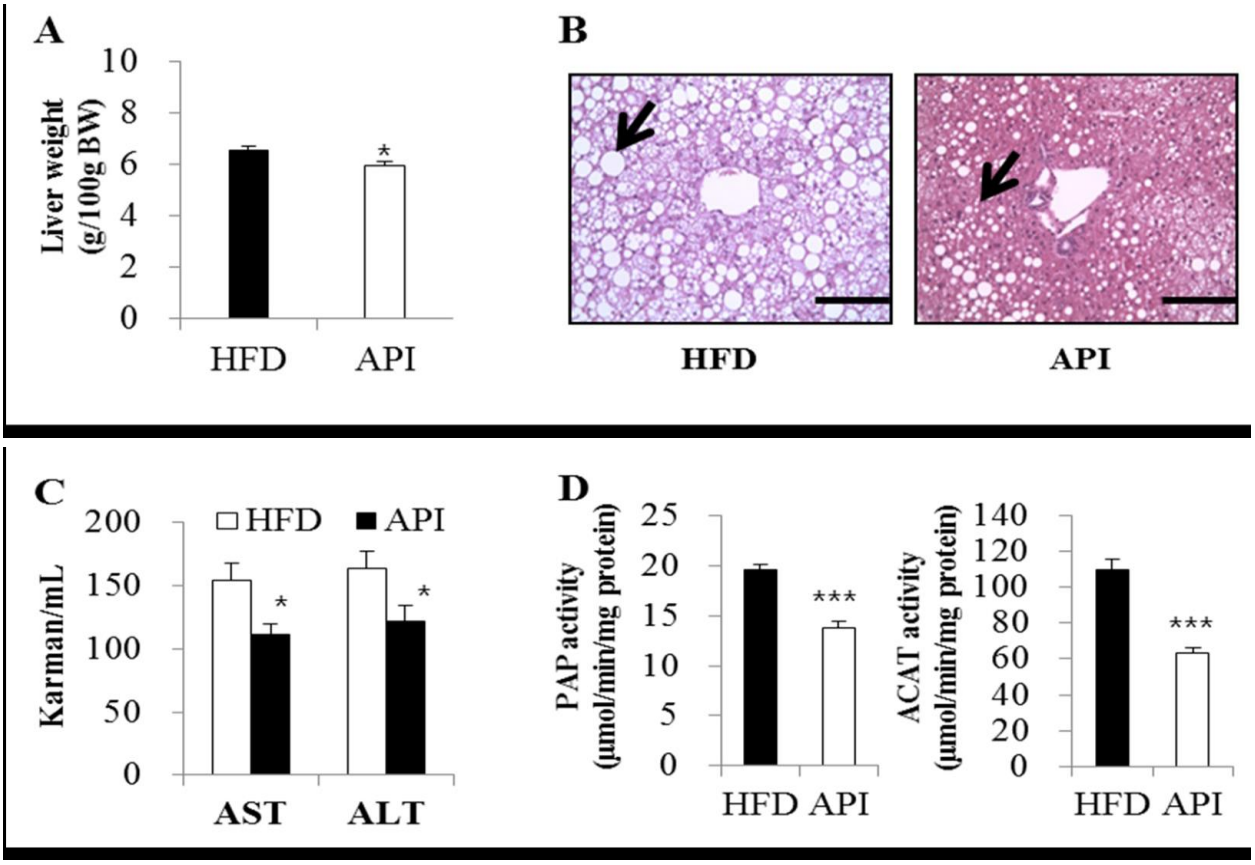

Fig 15: Chart view of antidiabetic activity

Effect of apigenin on liver weight (**A**), hepatic morphology (**B**), plasma transaminases activities (**C**) and activities of hepatic enzymes controlling the synthesis of triglyceride and cholesterol ester (**D**) in C57BL/6J mice fed a high-fat diet; ((**A**), (**C**), and (**D**)) Data are shown as the means ± S.E. Values are significantly different between the high-fat diet and apigenin groups according to Student's t-test: * $p < 0.05$, ** $p < 0.05$; (**B**) Original magnification ×200. Bar, 50 M. HFD: high-fat diet (20% fat, 1% cholesterol); API: HFD + 0.005% apigenin.

α-amylase:

Apigenin can suppress the activity of alpha-amylase depending on its action on carbohydrate binding regions of α-amylase enzymes that decrease the absorption of starch in to the body, and hence catalyze hydrolysis of α- 1,4 glucosidic linkages in the starch and polysaccharides and hence prevent hyperglycemia after meal. Inhibition of the α- amylase may occur via direct blockage of the active center at various sub- sites of the enzyme as a mention for another inhibitor. So, apigenin extract act as α-amylase inhibitor.

III. CONCLUSION

Although a large number of plants have been studied for their selective medicinal property, further more studies have not been carried out to the level of clinical trials because most of these research works are

independent and without industrial collaborations. One of the disadvantages of the previous studies is that most of them have not reported the cytotoxicity of the crude extracts or the purified fraction of the plant extracts. Some of the futures guidelines in this area of research are suggested below:

- We need to develop simple, economical multi-targeted green approaches that will be effective against multidrug resistant, plasmid elimination, virulence and pathogenicity reduction and so on.
- Herbal preparation with known efficacy of these plants in traditional system of medicine must be proved through scientific experiments and it should be tested both in animals and humans.

Therefore it is mandatory to invest enough money, time and energy to search an alternative plant based medicine. But to overcome the existing drawbacks, it is better not only to increase the list of the plants but also to re-evaluate and test the medicinal effect of reported plants. It is alleged that a superior therapeutically effective drug can be obtained from this plant source. Furthermore, the active participation of such natural custodians and practitioners of valuable knowledge is guaranteed in the generation of research focussing on screening programmes dealing with the isolation of bioactive principles and the development of new drugs which is the need of the hour.

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