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Development and Characterization of Herbal Nano Particulate herbal tablet of Euphorbia thymifolia l. for treatment of Diabetes

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ABSTRACT
The study on Euphorbia thymifolia focused on the authentication, extraction, and analysis of its bioactive compounds. The plant was collected and authenticated from the Western Ghats, and a pharmacognostic study revealed key morphological and microscopic features, including anomocytic stomata and fibrous roots. Various extracts were prepared using solvents like hexane, chloroform, ethyl acetate, ethanol, and water, with the ethanol extract yielding the highest percentage. Preliminary phytochemical screening indicated the presence of bioactive compounds such as alkaloids, glycosides, flavonoids, tannins, and steroids. Thin Layer Chromatography (TLC) further confirmed these findings, identifying several bioactive compounds in the ethanol extract. In vitro studies evaluated the extracts' antidiabetic potential, revealing significant alpha-amylase inhibition, particularly in the ethanol and hexane extracts, showing inhibitory activities of 73.33% and 70.00%, respectively. Subsequently, herbal nanoparticles were prepared using the nano-precipitation method with the ethanol extract of Euphorbia thymifolia, resulting in nanoparticles with a yield of 73.12%, a particle size of 95.6 nm, and a zeta potential of -19.5 mV. Stability studies over three months showed minimal changes in particle size, zeta potential, and entrapment efficiency. Additionally, nanoparticles containing an isolated compound (A4 fraction) demonstrated excellent stability, with particle size and zeta potential remaining stable and entrapment efficiency slightly improving. These results highlight the potential of Euphorbia thymifolia extract and its nanoparticles for pharmaceutical applications, particularly in antidiabetic therapy.

Keywords: Bioactive compounds, Alpha-amylase inhibition, Nano-precipitation method, Herbal nanoparticles, Nanoparticles stability.

1. INTRODUCTION

The study was aimed to investigate the qualitative and quantitative analysis of the major bioactive constituents present in plant Euphorbia thymifolia in ethanolic extract of whole parts of the plant. The plant is authentication from Botanical Survey of India Pune, by Scientist Mrs. Priyanka A. Ingale. The study was carried out in terms of aqueous extraction, total extractive values, qualitative and quantitative estimation of phytochemicals. The percentage value of yield extraction was found to be best was 14.15 %. The preliminary phytochemical analysis showed the presence of phenols, flavonoids, saponins, carbohydrates, terpenoids and glycosides. The spectroscopic evolution also did so far use the plant extract. It signifies those results revealed the presence of various bioactive constituents which could be exploited for their potential applications for medicinal purposes. Nano Particle in Diabetes- Improving oral bioavailability plays a key role in nano particle.
Oral nano delivery systems can not only protect antidiabetic phytocompounds from enzymatic and chemical degradation in GIT but also provide other benefits, such as avoidance of first-pass metabolism, improvement of pharmacokinetic and pharmacodynamic profile, fast onset of action, targeted drug delivery, sustained drug release, lower dose and dosing frequency, and fewer side effects

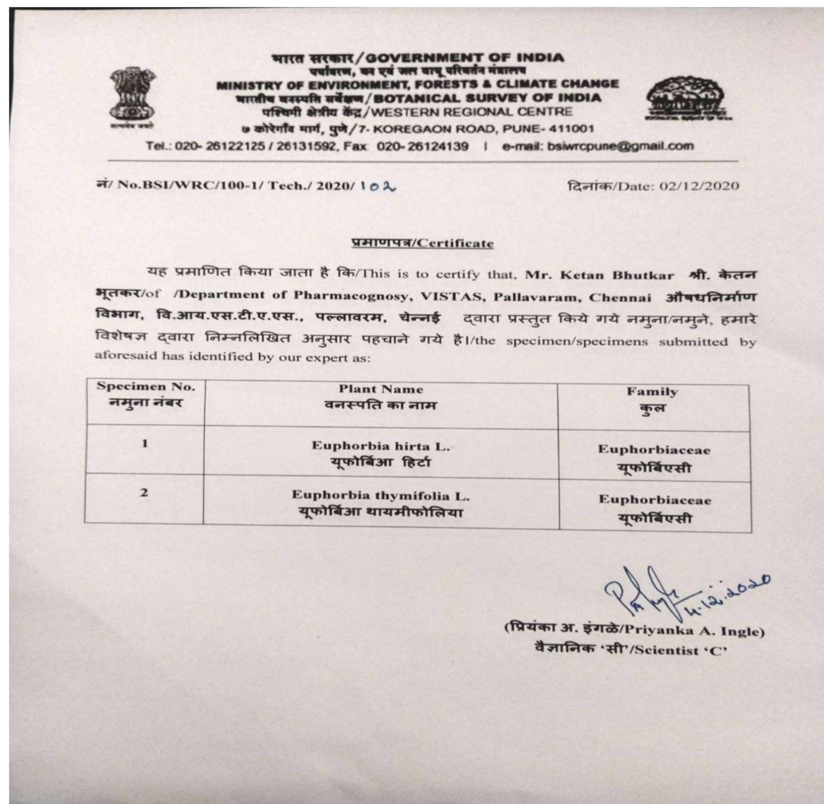
2. Experiment Work

1. **Collection and authentication of the plant's materials: -**
   Plant Material: The plant material was collected from hill area of western ghat near Bhor city district Pune Maharashtra on 26th September 2019 the plant material was identified by botanist.
   Authentication:

The first Specimen sample authenticated by Botanist – Dr. P. B. Kamble, Head of Botany Department A. T. College Bhor, dated 29th September 2019. Fresh plants of E. thymifolia were collected from BopdevGhat near under 10 km, area Pune Maharashtra India. and deposited the specimen for identified and authenticated at Botanical Survey of India Western Regional Office Pune. The Voucher specimens N / No. BSI/WRC/100-1/Tech./2020/102 by Scientiest Mrs. Priyanka A. Ingale for Comparison and authentication of plant species Euphorbia hirita and E. thymifolia are used.

Sample Sp. 01 Euphorbia hirita **Sample Sp. 02 Euphorbia thymifolia**

Figure 01: Specimen submitted to Botanical Survey of India at Regional office Pune



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प्रमाणपत्र/Certificate

यह प्रमाणित किया जाता है कि/This is to certify that, **Mr. Ketan Bhutkar श्री. केतन भूतकर**/of /**Department of Pharmacognosy, VISTAS, Pallavaram, Chennai औषधनिर्माण विभाग, वि.आय.एस.टी.ए.एस., पल्लावरम, चेन्नई** द्वारा प्रस्तुत किये गये नमुना/नमुने, हमारे विशेषज्ञ द्वारा निम्नलिखित अनुसार पहचाने गये है।/the specimen/specimens submitted by aforesaid has identified by our expert as:

Specimen No. नमुना नंबर	Plant Name वनस्पति का नाम	Family कुल
1	Euphorbia hirta L. यूफोर्बिआ हिर्टा	Euphorbiaceae यूफोर्बिएसी
2	Euphorbia thymifolia L. यूफोर्बिआ थायमीफोलिया	Euphorbiaceae यूफोर्बिएसी

4.12.2020

(प्रियंका अ. इंगळे/Priyanka A. Ingle)
वैज्ञानिक 'सी'/Scientist 'C'

Figure 02: Authentication letter By Botanical Survey of India Pune

2. Pharmacogenetic study: Morphology and Microscopy of plant.

2.1 Pharmacognostic Study: (1, 2, 5, 6)

The morphology, microscopy, powder characteristic, leaf constant was study. The physicochemical analysis, qualitative and quantitative phytochemical screening and TLC and IR spectroscopy study.

2.2 Morphology of plant: (2, 6,7)

For morphology study of plant select the different parts like root, stem, leaf, flower, and fruit and observe the size, shape, colour , odors and taste .

a. Plant **b. Stem** **c. Flower and fruit** **d. Root**

Figure 03: Parts of Plant Euphorbia thymifolia

- **Size :**E. thymifolia is annual prostate herb with 3-15 cm length as per climatic condition and irrigation its grown up to 30 cm
  - Leaves are arrange in opposite, elliptic, oblong or ovate.
  - The tip of plant apex is oval or rounded.
  - The leaf blades are oval-oblong or obliquely oblong with dentate margin
  - Stem is cylindrical, slender and spreading on the ground.
  - Fruits are ovoid globose, acutely 3-lobed and short hairy
- **Color:**
  - The pink color Petiole present in small and thin.
  - Stem branches radiating, pubescent and pink in colour.
  - Involucres axillary, solitary or in axil.
  - Cyathia in auxillary cluster.
  - Flowers are campanulate white in colour, fully mature flower convert to light pink in colour .
  - Fibrous root systems consist of many small roots.
- **Taste:**
  - Slight bitter

Figure 04: Size of Plant Euphorbia thymifolia

2.4 Ash value, extractive value and loss on drying values: [5, 6, 10]
Ash values such as total ash, acid insoluble ash, water soluble ash for Euphorbia thymifolia l. was 6.62, 1.06 and 1.82% respectively. Extractive values like alcoholic, aqueous, chloroform, ethanol respectively calculated. Loss on drying values was found to be 4.3%.

3. Extraction Of Plant Material: [15]
The selected plant material (crude drugs) Euphorbia thymifolia L. was collected from local place and dried in the shade. Then the dried material is pulverized in grinder. The powdered material was passed through 120 mesh sieves to remove fine powder and course powder was used for extraction.
Continuous Soxhlet extraction method used for extraction. (As the phytochemical literature revels that various metabolites are present in Euphorbia thymifolia L which need to separate by using different polarity solvent and continuous hot extraction method). Solvents of graded polarities like Hexane, chloroform, ethyl acetate and ethanol selected for extraction process with following objectives

- Hexane is the best solvent used to remove oily and fatty materials (Non polar)
- Chloroform useful for the removal of chlorophyll from leaves (slightly non-polar)
- Ethyl acetate extraction (phenolic complex compounds)
- Ethanol / methanol useful to remove all components (Polar complex compounds)
- After solvent extraction, water extraction of the plant material also useful.

3.1 METHOD: [16]
Each powered batch (200 gm. powder) loaded for extraction.Boiling temperature of solvent and all optimum laboratory requirements are maintained during extraction process. The completion of extraction was observed by following ways. Normally 72 cycles is considered as good criteria for extraction of the soluble constituents. Once confirmed the extraction completion in particular solvent, the remaining residual marc of crude drug was air-dried and further treated with the next step solvent. The extract was proceeding for further process to distill off solvent using a rotary evaporator apparatus (Buchi R-210 Rotavapor) at lowered (40-50°C) temperature and the concentrated extract was air-dried. The labeled and weighted extracts were stored in an airtight container for further analysis.

3.2 Following steps was followed for particular solvent extraction
1.Preparations of Hexane extract: [17]
Weight accurately crude drug course powder extracted with an adequate quantity of hexane using a soxhlet extractor at 60°-70°C. (Boiling point: 69°C)
2. Preparations of Chloroform extract:
After hexane extraction, the air-dried marc of crude drugwas repacked in a soxhlet apparatus and extracted with chloroform at 55°-65°C. (Boiling point: 61°C).
3. Preparations of Ethyl acetate extract:
After chloroform extraction, the air-dried marc of crude drugwas repacked in a soxhlet apparatus extracted with ethyl acetate at 60°-80°C. (Boiling point: 77°C).
4. Preparations of Ethanol extract:
After ethyl acetate extraction, the air-dried marc of crude drugwas repacked in a soxhlet apparatus and extracted with ethanol at 40-50°C. (Boiling point: 78°C).
5. Preparations of Aqueous extract:
After ethanol extraction, the air-dried marc of crude drug was macerated with distilled water containing chloroform (2.5ml/1000ml) for 48 hours with stirring. Then the resultant extract was filtered through a muslin cloth and the marc was separated. The filtrate was evaporated to dryness on hot plate at 45°C to get an aqueous semisolid extract.

4. Preliminary Phytochemical Screening: - [5,7,10,11,12]
Extracts were tested for the presence of active principles such as Terpenoid, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Proteins, Free Amino Acids, Carbohydrate and Vitamin C. Qualitative chemical test used to identify drug quality and purity. The identification, isolation and purification of active chemical constituents are depending chemical methods of evaluation.

5. Thin Layer Chromatography Of Extracted Compound
As for other methods in chromatography, the concept of separation relies on thin layer chromatography (TLC). The distinction depends on the relative affinity of the compounds of both phases. The compounds pass across the surface of the fixed phase during the mobile phaseThe degradation of the mixture is then accomplished. When the separation process is complete, the individual components of the combination appear on the plates as spots at their respective stages. Adequate identification methods classify their character and nature.

Steps involved in performing TLC of extracts

Prepared the slurry of adsorbent media (silica gel-G) in distilled water and poured the slurry on the TLC glass plates to obtain a thin layer. TLC plate was activated by heating in oven for 30min at 105°C.Dipping the capillary into the solution to be examined and applied the sample by capillary touched to the thin layer plate at a point about 2cm from the bottom. Air-dried the spot. The glass chamber for TLC should be saturated with mobile phase. Mobile phase was poured into the chamber and capped with lid. Allowed saturating about 30 min. After the saturation of chamber and spotting of samples on plate, it was kept in chamber. The solvent level in the bottom of the chamber must not be above the spot that was applied to the plate, as the spotted material will dissolve in the pool of solvent instead of undergoing chromatography. Allowed the solvent to run around 10-15cm on the silica plate. Plates were removed and were examined visually, under UV and suitable visualizing agent (Vanillin-H_2SO_4, Methanolic $FeCl_3$ solution) after that R_f was calculated by formula.

TLC- characterization of bioactive extract

After pharmacological evaluation all extracts, bioactive ethanol extract further evaluated by thin layer chromatography for determination of phytocomponents by following ways.

Table 01: TLC- characterization of bioactive extract

Sr. no.	Chemical constituent	Mobile Phase	Detection
1.	Alkaloids	n- butanol : Ethyl acetate: Formic acid : Water (30:50:10:10)	UV -365nm
2.	Glycoside	Ethyl acetate : Methanol : Water (100 : 16.5 : 13.5)	UV -365nm
3.	Flavonoid	Toluene : Ethyl acetate : Glacial acetic acid :Water (100:11:11:26)	Anisaldehyde – Sulfuric acid.UV -365nm
4.	Tannins	Ethyl acetate: Formic acid : Acetic acid : Water (100:11:11:26)	5% $FeCl_3$ in 0.1N HCl
5.	Steroids	Ethyl acetate : Methanol : Water (70 : 20 : 10)	Vanillin – Sulfuric acid.
6.	Terpenoids and Carotenoids	Cyclohexane : Ethyl acetate (75 : 25)	UV- 268nm
		Petroleum ether : Benzene (9 : 1)	UV- 254nm
7.	Triterpenoids	Chloroform : Glacial acetic acid Methanol :Water (60 : 32 : 12 : 8)	--
		Ethyl acetate : Glacial acetic acid : Formic acid : Water (100 : 11: 11 : 26)	Anisaldehyde – Sulfuric acid. UV – 254nm, 365nm

6. In-Vitro Study Of Enrich Extract[24, 26]

Sample :Herbalextracts **(using solvent Extraction)**

Sample Description: SampleEAQ,EHX, ECH,EET

EAQ- Water,**EHX-** Hexane, **ECH-**Chloroform,**EET-**Toluene Activity**:**

A. Alpha-Amylase Inhibition Assay

Alpha-amylase activity can be measured in-vitro by hydrolysis of starch in presence of alpha -amylaseenzyme. This process was quantified by using iodine, which gives blue colour with starch. The reducedintensity of blue colour indicates the enzyme-induced hydrolysis of starch in to monosaccharides. If thesubstance/extract possesses alpha -amylase inhibitory activity, the intensity of blue colour will be more.In other words, the intensity of blue colour in test sample is directly proportional to alphaamylaseinhibitoryactivity.

- Enzyme: (Type VI B: From porcine pancreas, 5 U/mg) [15.8 U/mg solid at pH 6.9]- Stored at 2-8^{0}C
- Substrate:Starch1%
- PositiveControl:Acarbose-StoredatRT-Glucobay(Bayerpharma,India)
- Sodiumdihydrogenorthophosphate(NaH2PO4.2H2O)-Himedia(RM-1255)-storedatRT
- Disodiumhydrogenphosphate(Na2HPO4.2H2O)-Himedia(RM-257)-storedatRT
- Indicator:Iodinesolution1%
- Instrument-VisibleSpectrometer.

Alpha-amylase activity was carried out by starch iodine method. 10 µL of α-amylase solution (0.025mg/mL) was mixed with 390 µL of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0)containing 1000µg/mlconcentration.Afterincubationat37°Cfor10min,100µL ofstarchsolution (1%) was added, and the mixture was reincubated for 1 h. Next, 0.1 mL of 1% iodine solution was added, and after adding 5mL distilled water, the absorbance wastakenat565nm. Sample, substrate and α-amylase blank determinations were carried out under the same reactionconditions. Inhibition of enzyme activity was calculated as (%) = (A-C)/ C X100, where, A=absorbanceofthesample, and C=absorbanceofcontrol (without starch).

B. In vitroevaluation of glucoseuptakebyyeastcells

Commercial baker's yeast was washed by repeated centrifugation (3,000×g, 5 min) in distilled wateruntil the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water.Various concentrations of extracts (5 mg/ml) were added to 1 ml of glucose solution (5.10, 25 mM) and further incubated for 10min at 37°C. Reaction was started by adding 100µl of yeast suspension, vortex and further incubation at 37°C for 60min. After 60min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. Metformin (100µg/ml) was taken as standard antidiabetic drug used. The percentage of increase in glucose uptake byyeastcells wascalculated usingthefollowingformula:

Increase in glucose uptakeActivity %=[(Abs control–Abs sample)/Abscontrol] ×100

Where,Abscontrolistheabsorbanceofthecontrolreaction(containingallreagentsexceptthetestsample) and Abs sample is the absorbance of the test sample. Absorbance was measured at 540 nmandall experiments werecarried outin triplicates.

7. Isolation Of Phytoconstituent

The column chromatographic isolation was targeted toward the isolation of bioactive secondary metabolite from bioactive ethanol extract.

i. Column Chromatography:

To isolate phytocomponents from bioactive ethanol extract mobile phase solvent used from range nonpolar to polar nature.

Procedure Details:

- Height of column : 38 cm.
- Diameter of column : 3.5 cm.
- Stationary phase: Silica gel for column chromatography (60-120#)
- Mobile phase: 1. n-Hexane
  2. Ethanol
  3. Chloroform
  4. Diethyl ether
  5. Water
- Flow rate: 6-8 drops per minute.
- Volume of each fraction: 100 ml (approximate)

8. Spectral Analysis

Spectral analysis of all fractions were done by using FTIR, H^1 NMR spectroscopy and Mass spectroscopy. The isolated extract after performing TLC, were found that steroids and flavonoids are the main constituents showing promising antidiabetic activity. The spectral characterisations are described below,

1. HPTLC of Isolated Compound

The sample was spotted in the form of bands of width of 6 mm with space between bands of 8.0 mm, with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F_{254} (5 cm ×10 cm) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm × 0.45 mm and scanning speed of 20 mm/sec was employed.

The linear ascending development was carried out in 10 cm×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using ethyl acetate: acetic acid: formic acid: water solvent in 10:0.5:0.5:1.0 (v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 15 min. The length of chromatogram run was 8 cm and development time was approximately 20 min. TLC plates were dried in a current of air with the help of a hair drier. After development densitometric scanning was performed on CAMAG thin layer chromatography scanner at 263 nm operated by WINCATS software version 1.4.2.

9.Preparation Of Herbal Nano Particle On & Characterization [18, 19]

1. Nanoprecipitation method

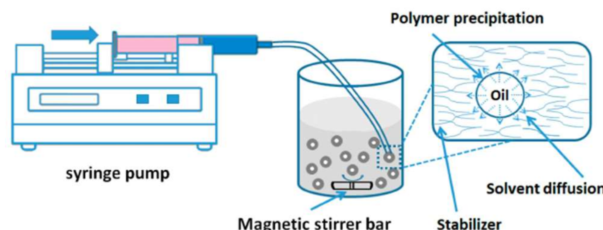


Figure 5: Preparation of Nano Particle by Nano precipitation method

Procedure

Plant extract about 2.5gm was dissolved in 15ml of acetone and ethanol in the ratio of 3:1 by sonication for 60 seconds. The resultant solution was then gradually injected about 1ml /minute with a syringe connected to a thin Teflon tube, into 25ml water containing PVA 1.5% w/v with continuous stirring by magnetic stirrer at 1000RPM. The resulting emulsion obtained was then diluted in 50ml PVA solution (0.2% in water) in order to minimize coalescence. Then mixture was continuously stirred at 500rpm for 6hr at room temperature to allow solvent evaporation and nanoparticle formation. The resultant Nano suspension was consequently cooled down to 18°C and lyophilized to obtained dry powder.

Table 2: A4 Fraction loaded herbal nanoparticles

Formulation	A4(mg)	PVA 1.5% (ml)	PVP 0.2%(ml)
F1	100	30	40
F2	100	25	50

2. Stability of Nanoparticles

The stability study of the F1 optimized nanoparticles over a three-month period, assessing particle size, polydispersibility index (PDI), zeta potential, and entrapment efficiency, indicates strong stability under storage conditions. The particle size remained consistent, with a slight reduction from -43.2 nm to -43.1 nm, suggesting minimal changes and no significant aggregation over time. The polydispersibility index (PDI) showed negligible variation, remaining around 0.115, indicating that the nanoparticle distribution was stable and uniform throughout the storage period. The zeta potential remained nearly constant, decreasing slightly from -28.9 mV to -28.6 mV, which reflects a stable surface charge and good colloidal stability. The entrapment efficiency was also consistent, with a slight increase from 81.12% to 81.9%, demonstrating that the nanoparticles maintained their drug-loading capacity well. Overall, the results show that the F1 optimized nanoparticles exhibit excellent stability in terms of particle size, PDI, zeta potential, and entrapment efficiency, making them suitable for extended storage and use.

10. Formulation of nanoparticle Tablet Dosage form

Preparation of Euphorbia thymifolia isolated compound loaded nanoparticles 250 mg tablet by direct compression method

Table 3: Formulation of nanoparticle Tablet Dosage form

Ingredients / Batch	Euphorbia thymifolia (mg)	HPMC K4M (mg)	HPMC K15M (mg)	MCC PH102 (mg)	Magnesium stearate (mg)	Talc (mg)
F1	200	8	-	36	2	4
F2	200	10	-	34	2	4
F3	200	20	-	24	2	4
F4	200	-	8	36	2	4

F5	200	-	18	26	2	4
F6	200	-	25	19	2	4
F7	200	30	-	14	2	4
F8	200	25	-	19	2	4
F9	200	28	-	26	2	4
F10	200	-	20	24	2	4
F11	200	-	30	14	2	4
F12	200	-	15	29	2	4

11. In vivo activity

Animals required

a. Species and Strain: Wistar rat

b. Age and Weight: Weight range: 20-30 g.(12-16 weeks)

c. Gender: Either sex

d. Number to be used (Year-wise breakups and total figures needed to be given in tabular form)

1. **For Acute toxicity**

Table 4: For Acute toxicity

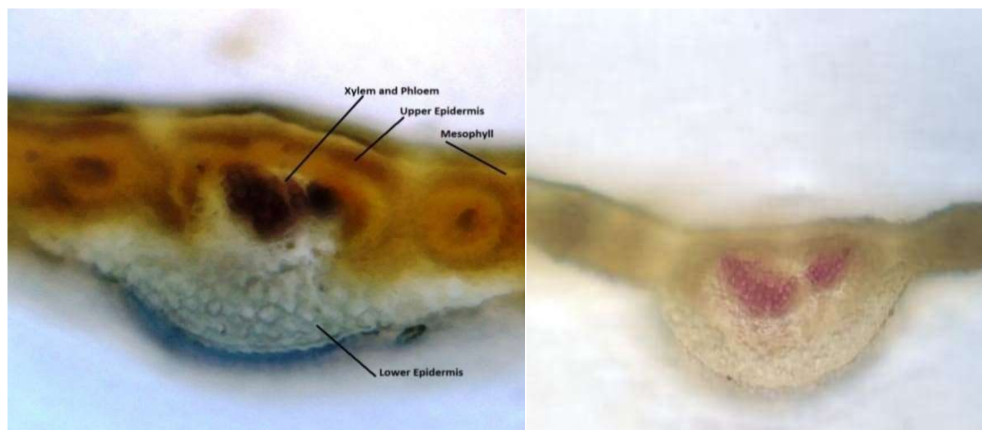
Sr. No	Name of group	Treatment (mg/kg)	No. of animals
1	Acute Toxicity	1000	6
		2000	6

2. In vivo streptozotocin induced antidiabetic activity

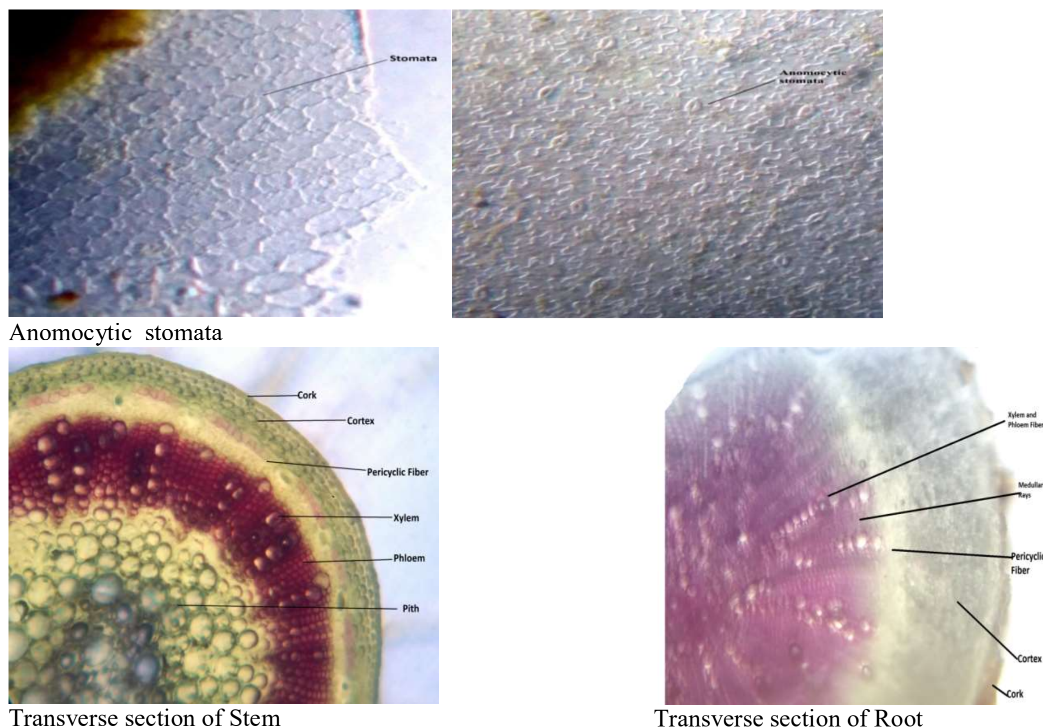
In vivo anti-diabetic study was performed for formulated nanoparticles of euphorbiaceous .the blood glucose level are tabulated in tablet. Initial blood glucose level before and after streptozotocin administration nanoparticle of Euphorbia thymifolia. The animal used for experiment will be 8-week old Swiss albino mice with weights ranging from 20 to 30 gram. The animals will be kept for 1 week in advance to adapt to the conditions of the cage before being treated. The mice will be placed in a cage that is given a husk base to absorb dirt from mice. During the adaptation period, the mice will be fed, watered and weighted daily. The mice are considered healthy when their weight increases or remains the same or decreases no more than 10%. The mice used for experiment were divided into 5 groups, containing 6 to 10 animals in each group. Before treatment, the mice fasted for 18 hours (ad libitum). Blood samples were drawn from each mouse‘s tail for measuring the first blood glucose levels (baseline). All mice then will treat intra peritoneally with 25mg/kg BW of streptozotocin, to elevate their blood glucose levels. Blood samples were collected from the tail vein of the overnight (12-15h) fasted mice and blood glucose level was determined on 0th, 7th, 14th and 28th day along with body weight and body temperature. If the blood glucose levels of mice > 200 mg/dL then the mice are considered to have hyperglycemia.

12. Result

1. Pharmacognostic study



Transverse section. of Leaf



Anomocytic stomata

Transverse section of Stem

Transverse section of Root

(Figure No. 06: Microscopically study of plant Euphorbia thymifolia
Transverse section of leaf, stem, root)

Leaf: TS of E. thymifolia shows a layer of the palisade cell.

- Anticlinal wall present in upper epidermis of lamina.
- Collateral vascular bundle present in meristem.
- Anomocytic stomata were present surface of E. thymifolia leaf.
- Trichome are absent over the surface of leaf E. thymifolia.
- Lactiferous ducts present inside the mesophyll area.

Stem: The TS of stem of E. thymifolia elongated cutinized epidermal cells are present & compactly arrangement, bearing comparatively more number of trichomes which are unicellular.

Cork: the cork layer present over the stem and root. 4-6 layered cortex in distinct endodermis and lignified pericyclic fibers. Xylem vessel arranged Radially with fibers and tracheids; lignified medullary rays are arrange in uni-biseriate manner.

- Oleoresin containing cells are present.
- Numerous primary xylem groups are seen towards the pith region.
- Pith is wide and parenchymatous and starch grains are present in the pith region.

Root: Phloem are narrow, contains latex duct followed by cambium and ring of xylem consisting of radially arranged vessels.

Flower And Fruit: Pubescent, petiole very minute, stipules fabricate with a pointed tip, inflorescence cyathium, involucres sub solitary, very short, axillaries especially in the crowded terminal branch lets, 4 ciliate, glands minute, stipulate with minute limb, ovary very small, tricarpellary, profusely hairy; stigma bifid; capsules erect, obtusely keeled, pubescent.

2. Ash value, extractive value and loss on drying values:

Table 5 : Ash value, extractive value and loss on drying values

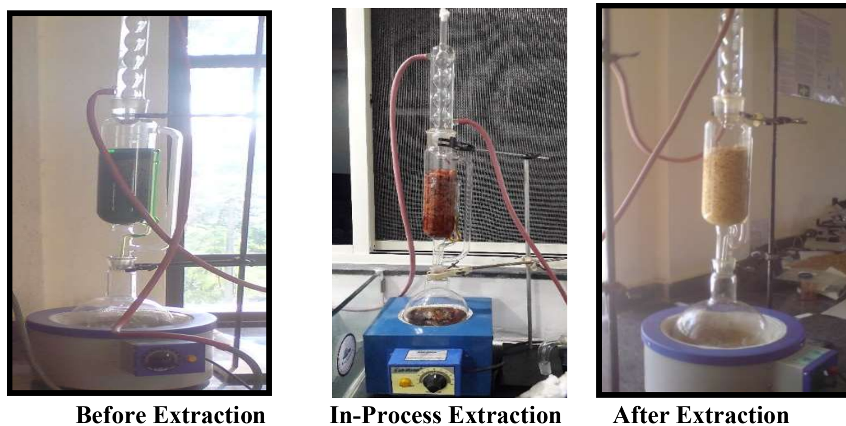
Sr. No	Evolution parameter	Result – value expressed in % W/W
A	**General Parameter**	
1	Loss on drying / Moisture content	3.19±0.04
2	Foreign organic matter	1.8±0.23
B	**Ash Value**	
1	Total Ash value	9.0±0.46

2	Acid insoluble ash	1.13±0.43
3	water soluble ash	3.5±0.27
4	Sulphated ash	2.29±0.33 %
C	**Extractive Value**	
1	Water soluble extractive value	7.86±0.62
2	Alcohol soluble extractive value	31.10±0.27

3. Extraction :

Tabel 6: Solvent used in extraction

Sr. No.	Extraction solvent used	Label
1.	Aqueous (Water) extract	EAQ
2.	Ethanol extract	EET
3.	Ethyl acetate extract	EEA
4.	Chloroform extract	ECL
5.	Hexane extract	EHX



Before Extraction **In-Process Extraction** **After Extraction**

Figure 06: Soxhlet extraction

Continuous hot Soxhlet extraction methods selected for extraction and reported characteristic of extract in table

Table 7: Characteristics and percent yield aerial parts of Euphorbia thymifolia L. extracts

Sr. No.	Extracts	Color	Nature	Percentage Yield (% W/W)
1.	Aqueous (Water) extract (EAQ)	Dark brown	Sticky powder	03.22%
2.	Ethanol extract (EET)	Green	Semisolid	17.16 %
3.	Ethyl acetate extract (EEA)	Dark Green	Jelly like	07.47%
4.	Chloroform extract (ECH)	Dark Green	Semisolid	15.15 %
5.	Hexane extract (EHX)	Green	Semisolid and sticky	08.61 %

4. Preliminary Phytochemical Screening

Table 8: Preliminary phytochemical screening of extracts

Extracts	Hexane	Chloroform	Ethyl acetate	Ethanol	Aqueous
Tests for carbohydrates					
Molish Test	-	+	-	-	-

Fehling Test	-	+	-	+	-
Benedict Test	-	+	-	+	-
Test for Monosaccharide					
Barfoed's Test	+	-	-	-	-
Test for Non-reducing polysaccharides					
Iodine Test	-	-	-	-	-
Test for Proteins					
Biuret test	-	+	-	-	-
Millions test	-	-	-	-	-
Tests for Steroids					
Salkowaski reaction	-	+	+	+	-
Libermann Burchard reaction	+	-	-	+	-
Libermann reaction	-	-	-	-	-
Tests for Terpenoids	+	+	-		-
Test for Glycosides					
Borntrager's Test	-	-	-	+	-
Killer- Killani Test	-	+	-	-	-
Test for Saponin					
Foam test	-	-	-	+	+
Tests for Flavonoids					
Shinoda test	-	-	+	+	-
Lead acetate Test	-	-	-	+	-
Sod-hydroxide Test	-	-	+	+	-
Tests for Alkaloids					
Meyers Test	-	+	-	+	-
Wagner's Test	-	-	-	-	+
Hager's Test	-	-	-	-	-
Dragendorff Test	-	+	+	+	-
Test for Tannins & Phenolic compounds					
$FeCl_3$	-	+	-	-	-
Lead acetate	-	+	-	+	-

+ Indicates presence of phytoconstituents, - Indicates absence of phytoconstituents

The all extracts were screened for the presence of various constituents. The result of this preliminary phytochemical examination is shown in above.

The result of phytochemical study on Euphorbia thymifolia L. revealed presence of primary metabolites as well as secondary metabolites such as carbohydrates, lipids, alkaloids, steroids, flavonoids, tannins and terpenoids after evaluation active extracts Ethanol further proceeds for TLC analysis to observe responsible potent phytochemicals.

5. TLC-Characterization. (Wagner 2004)

Solvent system used

Toluene: Ethyl acetate (9: 1) **(Ayurvedic Pharmacopoeia)**

Ethyl acetate: Methanol: Acetic acid (70:20:10) **(Stahl, 2005)**

Spray reagents

(i) Vanillin-Sulphuric acid reagent:

0.5 g vanillin is dissolved in 100 ml sulphuric acid- ethanol (40+10). Heated at 120^0c until maximum spot color intensity is reached.

Color observed - blue, blue-violet or pink colored spots.

(ii) Anisaldehyde-Sulphuric acid reagent: 0.5 ml of anisaldehyde was mixed with 10 ml glacial acetic acid, followed by 85 ml of methanol and 5 ml of concentrated sulphuric acid, in that order. The developed TLC plate was sprayed with reagent, heated at 100^0c for 5-10 minutes.
Color observed: blue, blue-violet or pink colored spots.

Thin Layer Chromatography of Euphorbia thymifolia L.extract

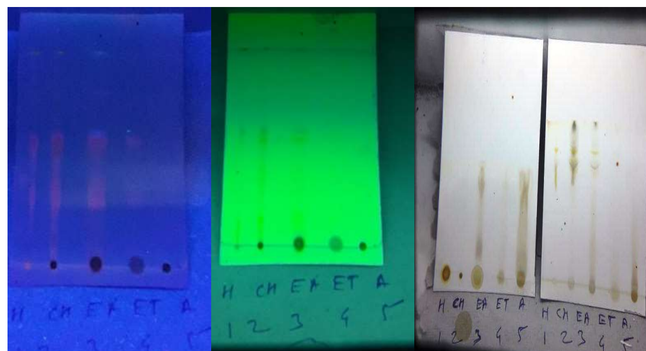


Figure 7: Observation under UV light

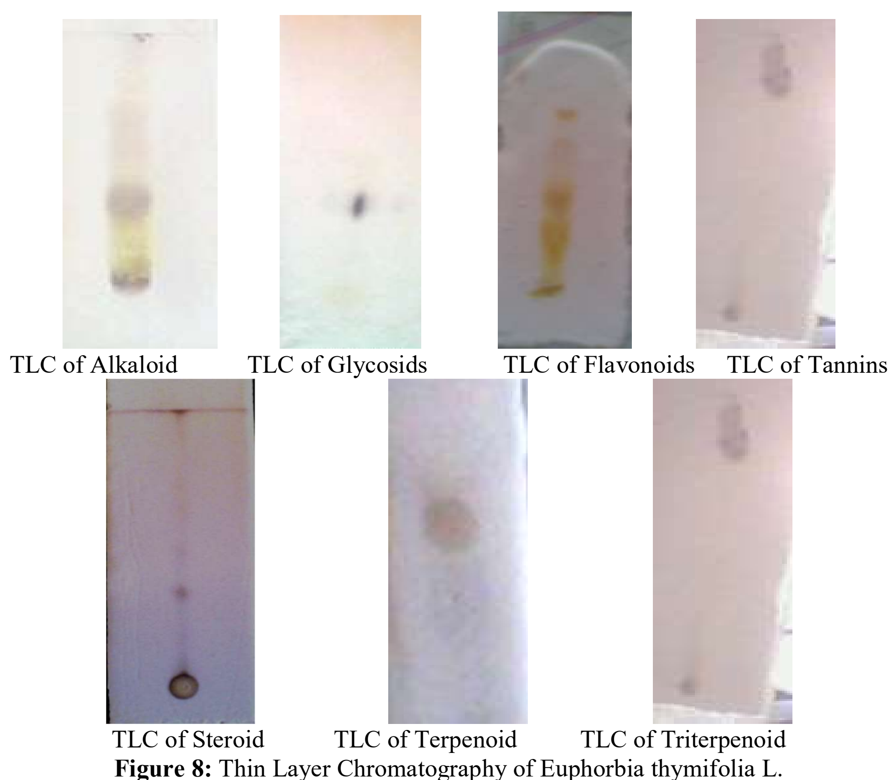


Figure 8: Thin Layer Chromatography of Euphorbia thymifolia L.

6. In-Vitro Study Of Enrich Extract

Table 9: The percent inhibition of extracts of plants on alpha amylase inhibitory assay

Sr. No.	SampleCode	Conc. µg/ml	OD	%inhibitionof alphaamylase
1	Blank	-	1.20	-
2	Standard–Acarbose	100	0.43	64.16
	Standard–Acarbose	500	0.31	74.16
	Standard–Acarbose	1000	0.11	**90.83**
3	EAQ	100	0.99	17.50

		500	0.95	20.83
		1000	0.62	**48.33**
4	EHX	100	0.68	43.33
		500	0.48	60.00
		1000	0.36	**70.00**
5	ECH	100	0.88	26.66
		500	0.81	32.50
		1000	0.60	**50.00**
6	EET	100	0.45	62.50
		500	0.43	64.16
		1000	0.32	**73.33**
7	EEA	100	0.42	49.85
		500	0.40	62.25
		1000	0.30	72.15

Conclusion: from above the extracts, **EHX and EET** showed good activity against amylase enzyme.

7. In vitroevaluation of glucoseuptakebyyeastcells
Observations

Table 10: Glucoseuptake Activity in %

Sr.no.	Treatment	OD	Increaseinglucoseuptake Activity%
1	Control5mM	0.91	-
2	Control10mM	0.71	-
3	Control25mM	0.31	-
4	Standard5 mM	0.17	81.31
5	Standard10 mM	0.15	78.87
6	Standard25 mM	0.14	**54.83**
7	EAQ5mM	0.85	06.59
8	EAQ10mM	0.68	04.22
9	EAQ25mM	0.24	**22.58**
10	EHX5mM	0.81	10.98
11	EHX10mM	0.60	15.49
12	EHX25mM	0.18	**41.93**
13	ECH5 mM	0.68	25.27
14	ECH10 mM	0.52	26.76
15	ECH25 mM	0.21	**32.25**
16	EAE 5mM	0.59	22.36
17	EAE 10mM	0.48	19.24
18	EAE 25mM	0.23	**27.84**
19	EET5mM	0.61	32.96
20	EET10mM	0.43	39.43
21	EET25mM	0.17	**45.16**

Conclusion: According to table:At the concentration of 5mg/ml,The **EHX and EET** showed good activity as compared to standard.

8. Column Chromatography

Table 11: Details of Gradient solvent system in column chromatography for the ethanolic extract

Solvent used	Ratio	Fraction Code	Colour	% Yield
Hexane	100%	A1	Dark brown	1.4
Hexane: Ethanol	7:3	A2	Brown	1.6
Hexane: Ethanol	5:5	A3	Brown	1.64
Hexane: Ethanol	2:8	**A4**	**Dark brown**	1.72
Hexane: Ethanol	6:4	**A4**	**Dark brown**	1.70

Ethanol: Chloroform	7:3	A6	Yellowish-brown	1.67
Ethanol: Chloroform	5:5	A7	Yellowish-brown	1.22
Chloroform	100%	A8	Yellow	1.7
Chloroform: Diethyl ether	7:3	A9	Yellow	0.7
Chloroform: Diethyl ether	5:5	A10	Faint yellow	0.5
Diethyl ether	100%	A11	Brown	0.5
Diethyl ether: Water	7:3	A12	**Dark Brown**	1.4
Diethyl ether: Water	5:5	A13	Yellow	1.7
Water	100%	A14	Faint yellow	1.1

Above isolated fractions are concentrated and evluated for TLC analysis. The fraction number A4 and A5 showed better sepration and identification of flavonoid derivative as well as polyphenolic copound.
So, both fractions are charactrised for advance structural ellucidation techniques for detail charactristics.

9. TLC Analysis of Fraction A4, and Standard flavonoid Quercetin.

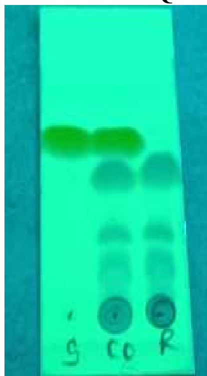


Figure 10: TLC A4 S- Sample A4 , Co- Sample A 5 and R – Reference

Table 12: TLC- Characterization of bioactive extract and reference standard flavonoid.

Mobile Phase	Visualization / Detection	Observed Rf-value	Reference Rf value
Toluene : Ethyl acetate : Glacial acetic acid :Water (100:11:11:26)	Anisaldehyde – Sulfuric acid.	S-A5- 0.63 Co-A4- 0.62	0.62

Above analysis and comparison with standard flavonoid compound Quercetin Rf value, express complete isolation and identification of flavonoid derivative is from fractions. Therefore, Bioactive A4 and A 5 fraction processed for structural analysis.

10. Column Chromatography

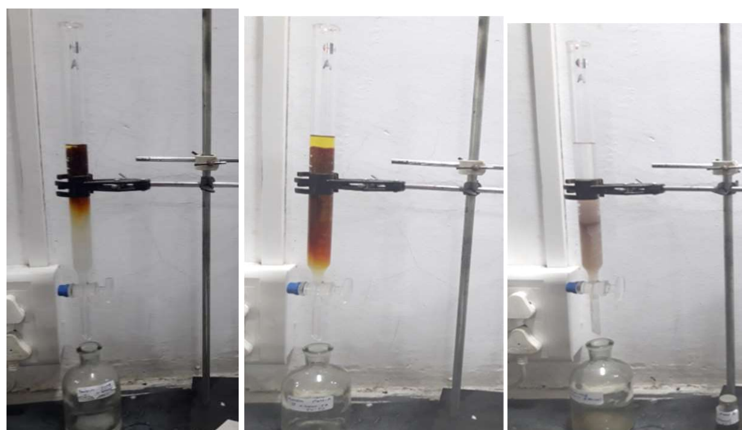


Figure 9: Column Chromatoghraphy Analysis

Details of Gradient solvent system in column chromatography for the ethanolic extract

Table 13: Details of Gradient solvent system in column chromatography for the ethanolic extract

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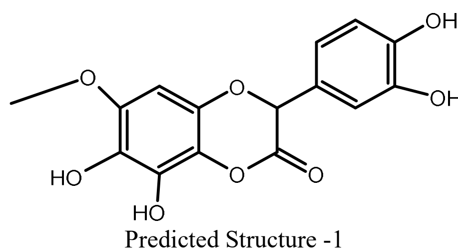
Hexane: Ethanol	5:5	A3	Brown	1.64
Hexane: Ethanol	2:8	**A4**	**Dark brown**	1.72
Hexane: Ethanol	6:4	**A4**	**Dark brown**	1.70
Ethanol: Chloroform	7:3	A6	Yellowish-brown	1.67
Ethanol: Chloroform	5:5	A7	Yellowish-brown	1.22
Chloroform	100%	A8	Yellow	1.7
Chloroform: Diethyl ether	7:3	A9	Yellow	0.7
Chloroform: Diethyl ether	5:5	A10	Faint yellow	0.5
Diethyl ether	100%	A11	Brown	0.5
Diethyl ether: Water	7:3	A12	**Dark Brown**	1.4
Diethyl ether: Water	5:5	A13	Yellow	1.7
Water	100%	A14	Faint yellow	1.1

Above isolated fractions are concentrated and evluated for TLC analysis. The fraction number A4 and A5 showed better sepration and identification of flavonoid derivative as well as polyphenolic copound.
So, both fractions are charactrised for advance structural ellucidation techniques for detail charactristics.

11. SPECTRAL ANALYSIS:
Compound 1-
Predicted Molecular Formula of the compound 1- $C_{15}H_{12}O_8$
Mol. wt. 320.25
3-(3,4-dihydroxyphenyl)-7,8-dihydroxy-6-methoxy-1,4-benzodioxin-2(3H)-one
M. P- 329 ^{0} C
B.P- 392^{0} C
Colour- Brown
R.I- 1.4729

A. FT-IR SPECTRA



Predicted Structure -1

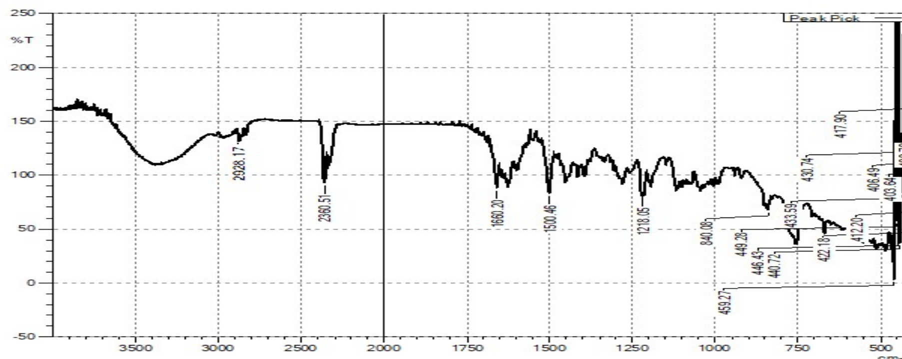
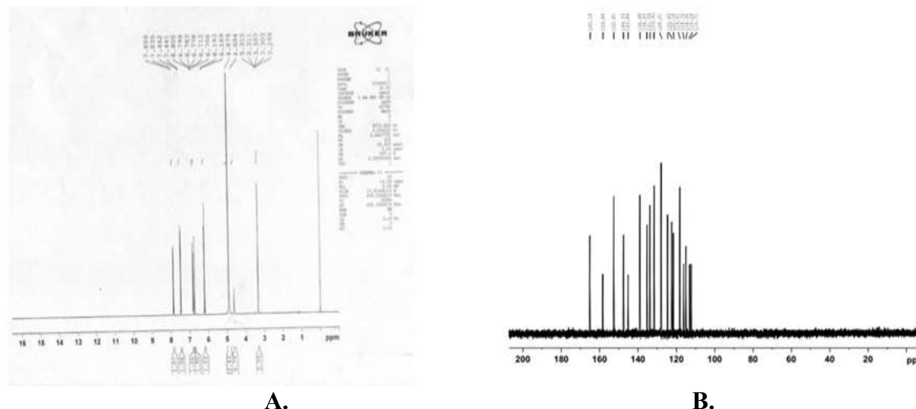


Figure 11: IR Spectra of isolated compound

Table 14: IR Interpretation of isolated compound

Reported Ir ranges(cm^{-1})	Observed Ir ranges(cm^{-1})	Functional group
2500 – 3300 (broad) alkene	2928.17	O-H stretching
1650 – 1750 (strong) aldehyde	1660.0	C=O stretching
2300-2400	2360.51	C≡O
1500-1430	1500.46	C=C stretching
1000-1300	1218.05	C-O
Below 900	840.08,459.27,449.28,446.43, 440.72,422.18,412.20,430.74	=C-H

B.13C NMR and 1-HNMR spectrum of the isolated compound



A. **B.**

Figure 12. 13C NMR and 1H NMR spectrum of the isolated compound

The 13C NMR spectrum of the isolated compound I showed nine prominent signals. A peak at
1. 163.7 was assigned to the corner function (C-2) of the compound.
2. At C-7, a downfield signal at
3. 163.1 revealed the presence of a Hydroxyl function. C-8, C-3, C-4 A, C-6, C- 5, C-4 and C-8 A were assigned to other signals at
4. 103.4, 112.3, 113.1, 114.5, 130.6, 146.0 and 157.2 respectively.

The 1HNMR spectrum of the isolated compound showed nine prominent signals. A peak at
1H NMR: δ 3.31 (3H, s),
6.16 (1H, s),
6.19 (1H, s),
6.78 (1H, dd, J = 2.7, 0.5 Hz),
6.79 (1H, dd, J = 8.5, 0.5 Hz),
6.80 (1H, dd, J = 8.5, 2.7 Hz).

C. LCMS of the isolated compound:

The LC MS showed a molecular ion peak at m/z 323 (M]+ and was in match with the proposed structure with C15H12O8. Hence, it was concluded through spectroscopic studies that the compound I could be characterised as flavonoid derivative.

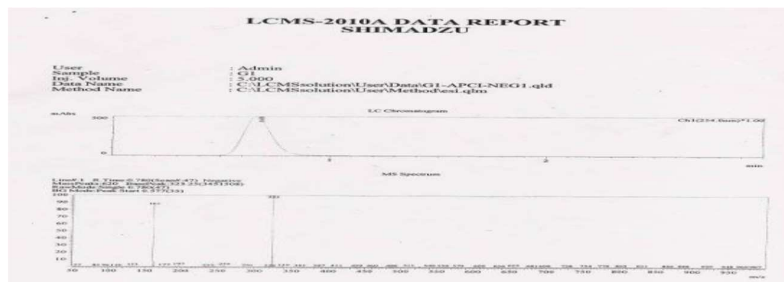


Figure 13: LCMS of the isolated compound

D. Ultraviolet Spectroscopy of Isolated compound

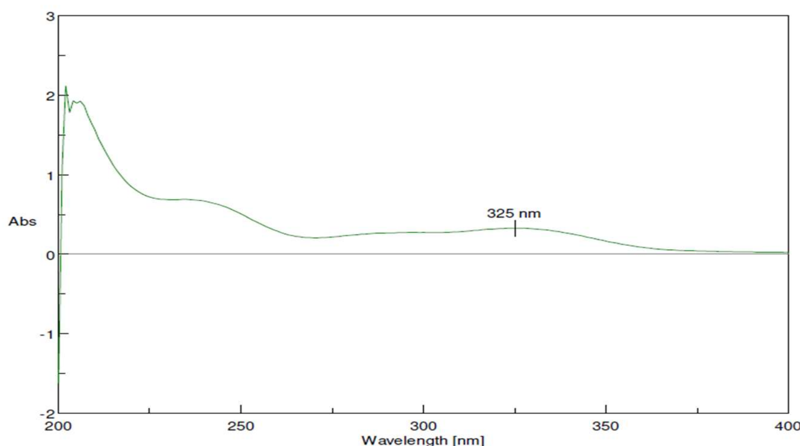


Figure 14: U.V. Spectroscopy of the isolated compound

Table 15. Absorbance of U.V. Spectroscopy of the isolated compound

Concentration	Absorbance
0	0
0.2	0.052
0.4	0.1064
0.6	0.147
0.8	0.1957
1	0.245

11. HPTLC of Isolated Compound

Standard Quercetin Solution Preparation: Standard solution of Quercetin was prepared by dissolving 10 mg of Quercetin RS in methanol to get 1000 µg/ml solution. From this 1 ml was further diluted to 10 ml to get 100 µg/ml solution. This solution was applied on TLC plate (2, 4, 6, 8, 10, 12 µl, 14 µl, 16 µl and 18 µl) to get concentration range from 200- 1800 ng/spot)

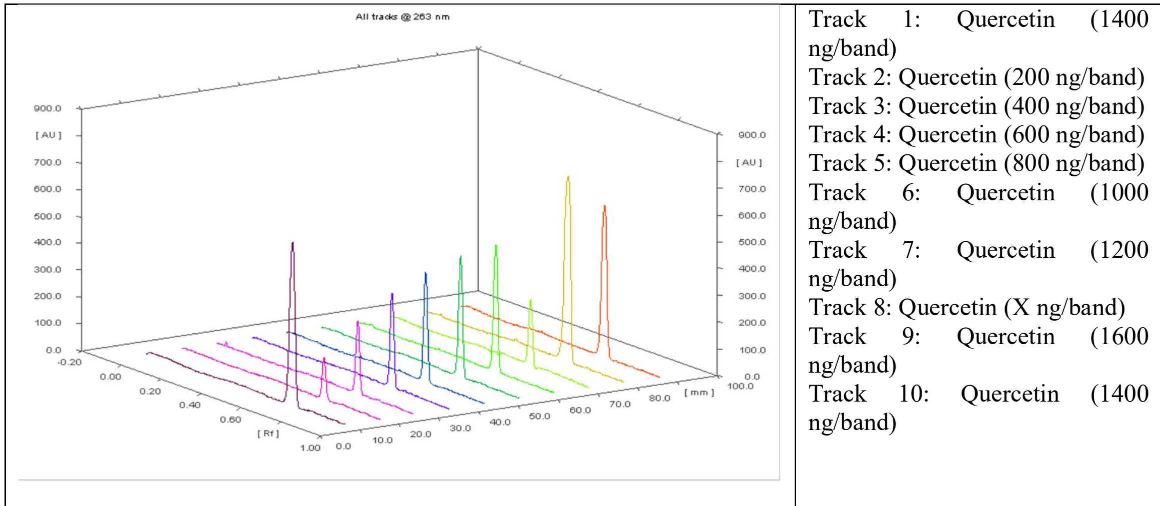
Sample Solution Preparation: Sample was dissolved in 10 ml of methanol. 2 µl volume was applied on the plate.

1 2 3 4 5 6 7 8 9 10

Figure 15: HPTLC Plate Seen at Visible light

1 2 3 4 5 6 7 8 9 10

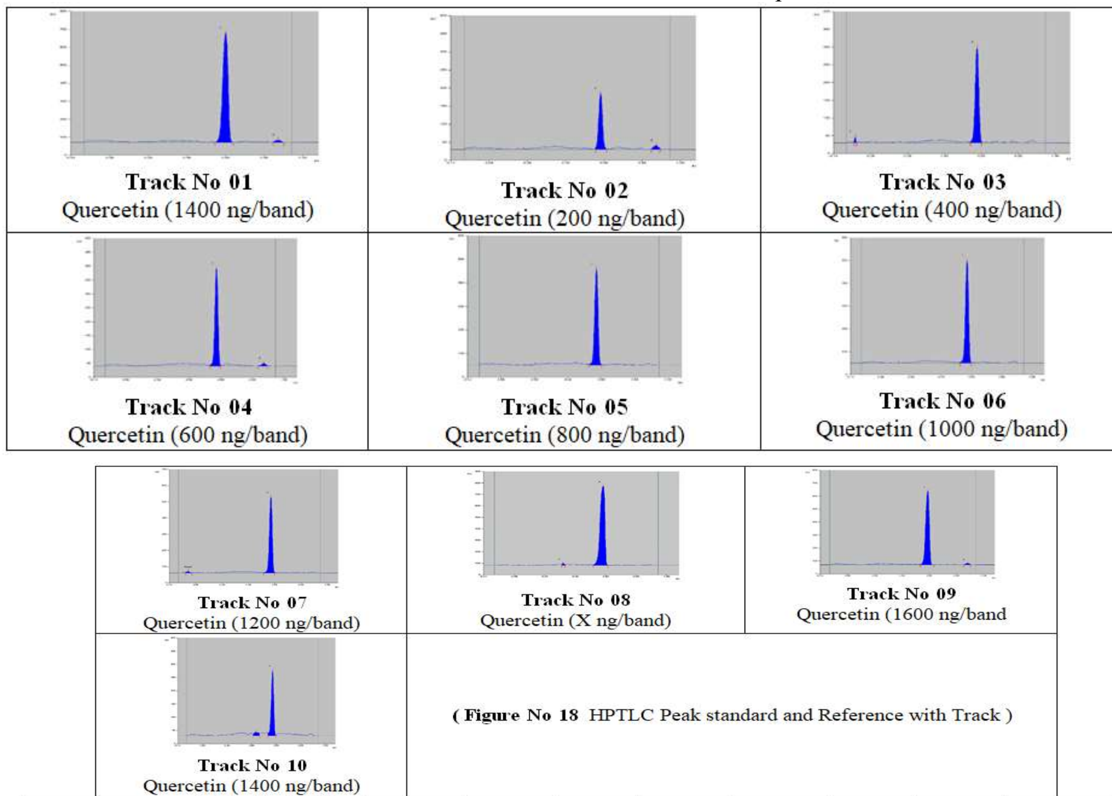
Figure 16: HPTLC Plate Seen at 254nm



Track 1: Quercetin (1400 ng/band)
Track 2: Quercetin (200 ng/band)
Track 3: Quercetin (400 ng/band)
Track 4: Quercetin (600 ng/band)
Track 5: Quercetin (800 ng/band)
Track 6: Quercetin (1000 ng/band)
Track 7: Quercetin (1200 ng/band)
Track 8: Quercetin (X ng/band)
Track 9: Quercetin (1600 ng/band)
Track 10: Quercetin (1400 ng/band)

Figure 17: D Densitogram (All tracks scanned at 263 nm))

Table 16: HPTLC of standard and isolated compound



(**Figure No 18** HPTLC Peak standard and Reference with Track)

Track No	Name	Track No.	Volume Applied	Conc (ng/band)	Start Position	Start Height	Max Position	Max Height	End Position	End Height	Area AU
1	Quercetin	Track 1	14 µL	1400	0.59 Rf	3.2 AU	0.65 Rf	570.0 AU	0.70 Rf	0.1 AU	10509.1
2	Quercetin	Track 2	2 µL	200	0.61 Rf	3.1 AU	0.63 Rf	149.5 AU	0.67 Rf	0.1 AU	2312.0
3	Quercetin	Track 3	4 µL	400	0.60 Rf	1.8 AU	0.63 Rf	273.0 AU	0.67 Rf	1.7 AU	4221.6
4	Quercetin	Track 4	6 µL	600	0.59 Rf	3.2 AU	0.62 Rf	545.2 AU	0.67 Rf	3.6 AU	5530.2
5	Quercetin	Track 5	8 µL	800	0.59 Rf	3.2 AU	0.62 Rf	401.2 AU	0.67 Rf	3.5 AU	7045.5
6	Quercetin	Track 6	10 µL	1000	0.58 Rf	2.4 AU	0.62 Rf	451.2 AU	0.67 Rf	1.5 AU	7785.6
7	Quercetin	Track 7	12 µL	1200	0.57 Rf	1.2 AU	0.62 Rf	469.5 AU	0.67 Rf	3.2 AU	9021.6
8	Sample	Track 8	2 µL	X	0.59 Rf	9.3 AU	0.62 Rf	247.2 AU	0.67 Rf	0.2 AU	4312.3
9	Quercetin	Track 9	16 µL	1600	0.58 Rf	6.9 AU	0.64 Rf	694.0 AU	0.68 Rf	1.9 AU	11931.2
10	Quercetin	Track 10	18 µL	1400	0.60 Rf	0.9 AU	0.65 Rf	599.8 AU	0.69 Rf	0.3 AU	12235.6

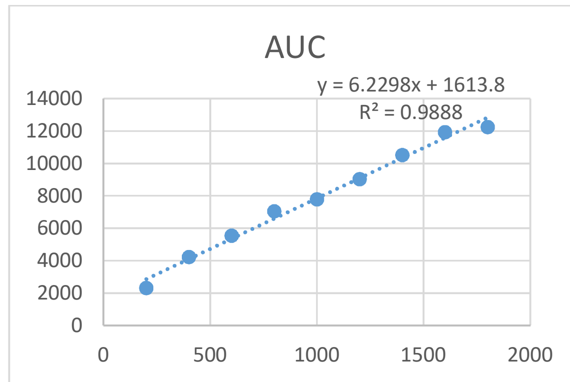


Figure 19 : Plot of Area against Quercetin Concentration

Regression Equation - Y = 6.2298X + 1613.8 (Y = mx + c)

Quantification:
2 µL of sample applied. It contains 433.15ng of Quercetin through linear equation
Therefore 10 ml (10000 µL) of diluted sample contains = 4081.710ng = 0.4332µg of Quercetin.

12. Characterization of extract loaded nanoparticles

i. Production yield of nanoparticles

Nanoparticles were collected and weighed accurately. The percentage (%) yield was then calculated using formula given below.

$$\% \text{ Yield} = \frac{\text{Mass of nanoparticles obtained}}{\text{Total weight of drug and polymer}} \times 100.$$

ii. Production yield of nanoparticle

Table 17: Production yield of EET Extract loaded nanoparticle

Formulation	**Production yield (%)**
EET	73.12

A. Particle Size and Zeta Potential

Formulation	**Particle size (nm)**	**PDI**	**Zeta potential (mV)**
EET	95.6	0.316	-19.5

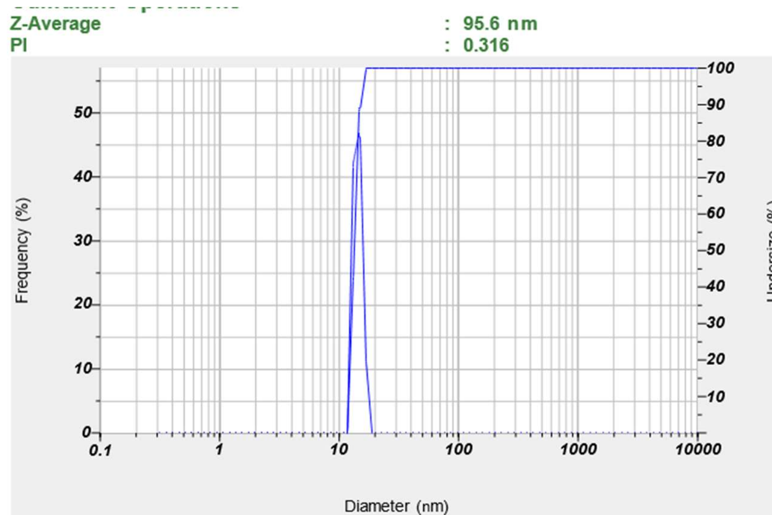


Figure 21: particle size of EET extract loaded nanoparticles

Zeta Potential (Mean) : -19.5 mV
Electrophoretic Mobility Mean : -0.000114 cm^2/Vs

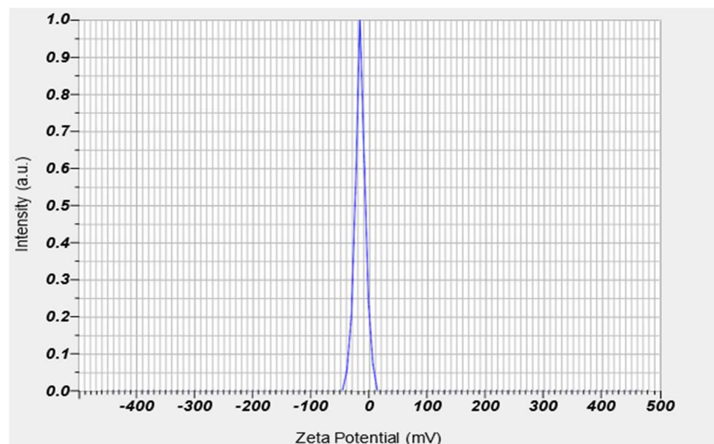


Figure 22: Zeta potential of EET extract loaded nanoparticles

B. Scanning electron microscopy and Surface Morphology

Particle shape and its arrangement inside the formulation can be unfolded by scanning electron microscopy (SEM). Scanning electron microscopy was used to examine the surface morphology of nanoparticles. The SEM graphs are represented below. The morphology for prepared nanoparticles was analyses by using a Hitachi S-4700 SEM (scanning electron in Hitachi Company, Japan).

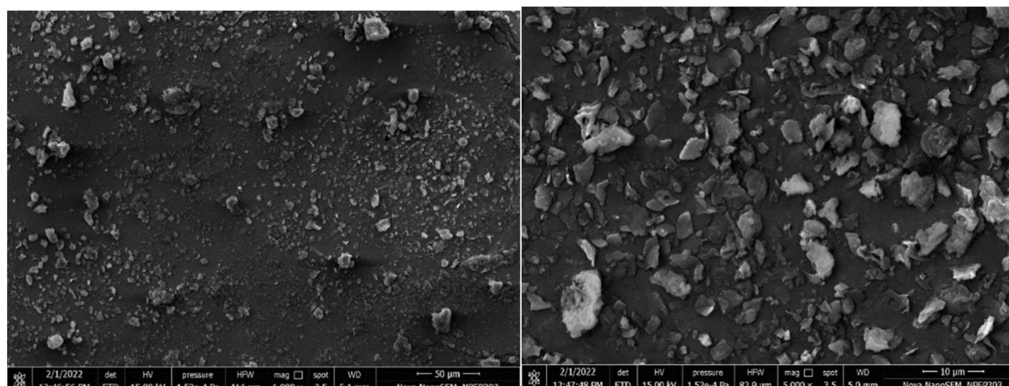


Figure 23: Scanning electron microscopy and Surface Morphology of EET extract loaded nanoparticles

C. Drug entrapment efficiency

Percent entrapped drug and free drug percentage of nanogel dispersion was determined using the UV spectrophotometric. The entrapment efficiency was found to be 69.12 % which provides optimum availability of drug at site without any side effects.

Table 18: Entrapment efficiency of EET extract loaded nanoparticles

Formulation	**Entrapment efficiency (%)**
EET	69.12

12. Transmission Electron Microscopy Of Extract

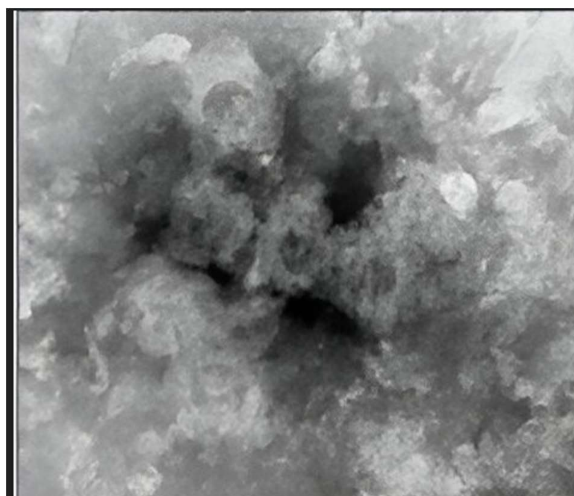


Figure 24: TEM of EET extract nanoparticles

D. Stability of nanoparticles

The stability study of EET extract-loaded nanoparticles over a period of three months demonstrated excellent results across key parameters, including particle size, zeta potential, and entrapment efficiency. The particle size remained almost constant, with only a minimal decrease from 95.6 nm to 95.4 nm, indicating that the nanoparticles did not undergo significant aggregation or growth. Similarly, the zeta potential exhibited remarkable stability, changing only slightly from -19.5 mV to -19.4 mV, which reflects good surface charge stability and suggests that the nanoparticles maintained their dispersion without clustering. The entrapment efficiency showed a minor reduction from 69.12% to 68.12%, with only a 1% loss over the three months, signifying that the drug retention within the nanoparticles remained high. Overall, the study concludes that the EET extract-loaded nanoparticles exhibit robust stability, making them suitable for long-term use and further development.

Table 19: Stability of EET extract loaded nanoparticles

Storage time	**“0” Month**	**“1” Month**	**“2” Month**	**“3” Month**
Particle size (nm)	95.6±0.35	95.6±0.46	95.6±0.74	95.4±0.02
Zeta potential (mV)	-19.5±0.14	-19.5±0.35	-19.5±0.36	-19.4±0.34
Entrapment efficiency (%)	69.12±0.65	69.12±0.89	69.12±0.29	68.12±0.78

13. Characterizations of Nanoparticles of isolated compound

The prepared nanoparticle of Isolated A4 Fraction was subjected for different evaluations parameters.

i. Production yield of nanoparticles

It was discovered that as the concentration of polymer increased, the yield increased.

Table 20. Production yield of Euphorbia thymifolia extract loaded nanoparticle

Formulation	**Production yield (%)**
F1	72.41
F2	69.12

ii. Particle size determination by Zeta sizer

The particle size of nanoparticle made from Isolated A4 Fraction was determined. The nanoparticle’s particle size was determined to be between a ranges of 1-100nm.

Table 21: particle size and zeta potential of F1 and F2 loaded nanoparticles

Sr. No.	**Sample**	**Nanoparticle Size (nm)**	**PDI**	**Zeta Potential (mV)**
1	F1	43.2 ± 0.11	0.115	-28.9
2.	F2	82.5±0.12	0.121	-21.0

Values are shown as the mean ± standard deviation; n=5.

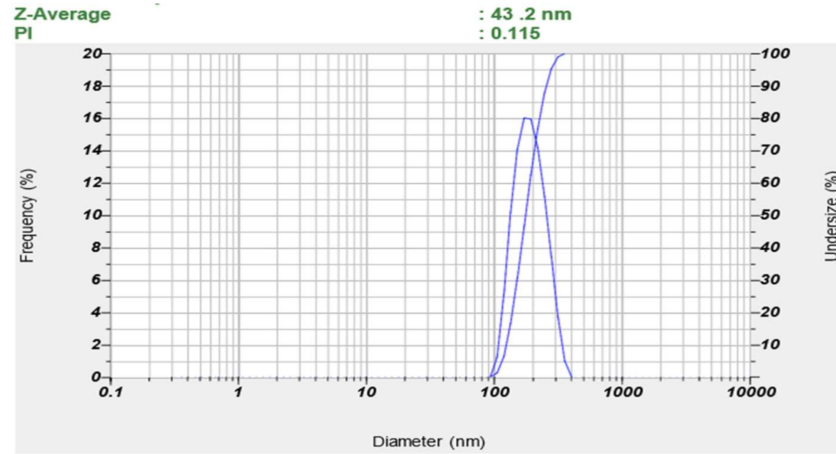


Figure 25. Particle size of F1 loaded nanoparticles

Zeta Potential (Mean) : -28.9 mV
Electrophoretic Mobility Mean : -0.000202 cm^2/Vs

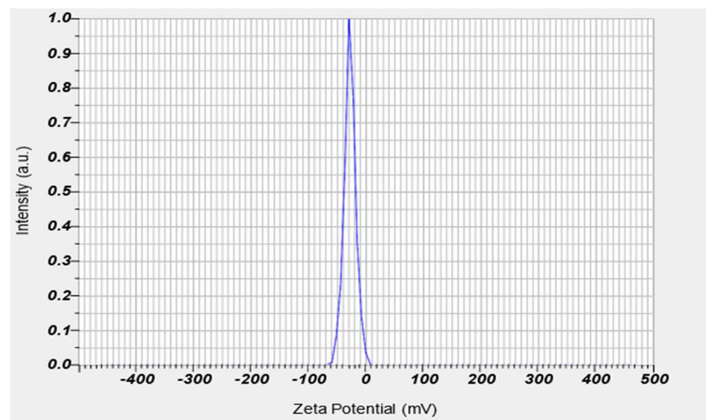


Figure 26. Zeta potential of F1 loaded nanoparticles

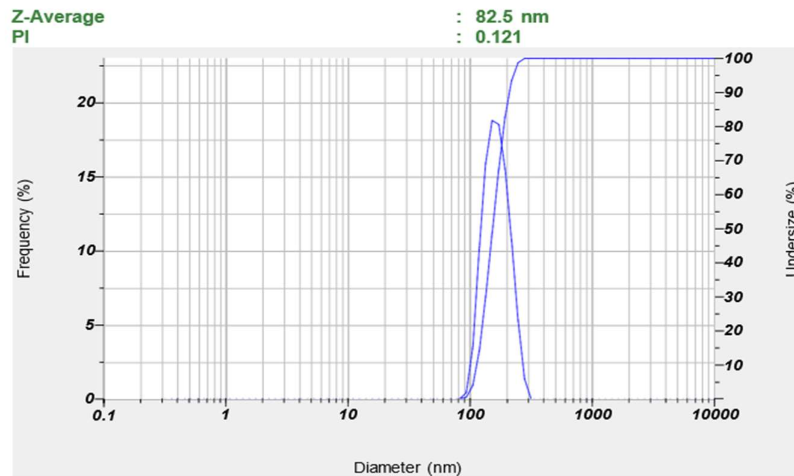


Figure 27. Particle size of F2 loaded nanoparticles

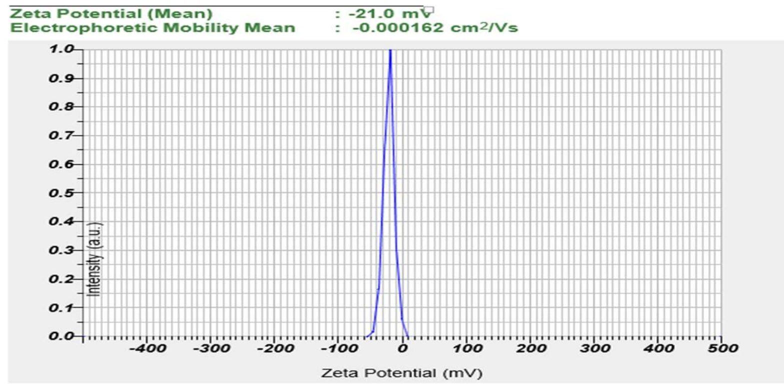
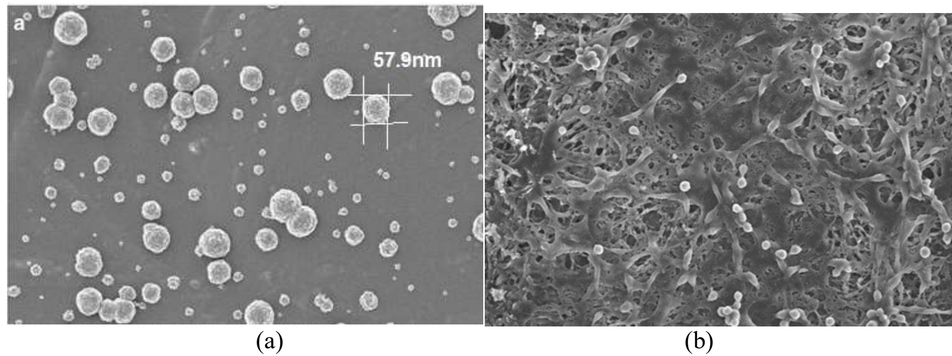


Figure 28. Zeta potential of F1 loaded nanoparticles

iii. Scanning electron microscopy

Scanning electron microscopy was used to examine the surface morphology of PVA nanoparticles. A high number of nanoparticles with a roughly spherical form were present, and they will split from one another. SEM picture of a freeze-dried PVA nanoparticle with a longer cross-linking period, showing a tiny, spherical nanoparticle with a small size.



(a) (b)

Figure 29. (a) Scanning electron micrograph of F1 nanoparticles and (b) freeze-dried F1 nanoparticles

iv. Drug entrapment efficiency

The entrapment efficiency of A4l oaded nanoparticle was observed 81.12 %, indicating higher drug entrapment efficiency.

Table 22. % Entrapment efficiency of Euphorbia thymifolia extract loaded nanoparticle

Formulations	**Entrapment efficiency (%)**
F1	81.12
F2	79.12

v. Transmission electron microscopy

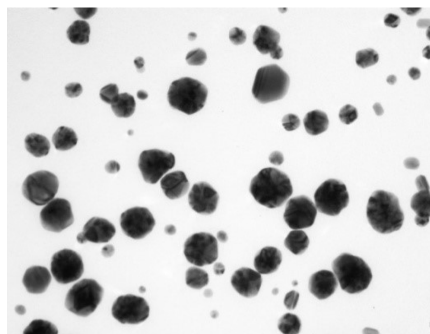


Figure 30. F1 optimized batch of TEM

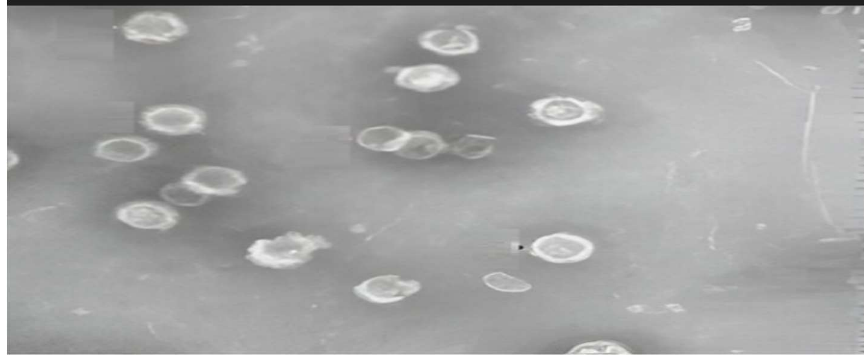


Figure 31: F2 optimized batch of Tem) Image

vi. Stability of Nanoparticles of F1

Table 23. Effect of storage on particle size, zeta potential and entrapment efficiency of F1 Optimized Nanoparticles

Storage time	"0" Month	"1" Month	"2" Month	"3" Month
Particle size (nm)	-43.2±0.02	-43.2±0.01	-43.2±0.31	-43.1±0.05
polydispersibility index	0.115±0.03	0.115±0.05	0.114±0.06	0.114±0.145
Zeta potential (mV)	-28.9±0.04	-28.9±0.08	-28.9±0.58	-28.6±0.87
Entrapment efficiency (%)	81.12±0.09	81.12±0.07	81.120.79	81.9±0.53

(n=3). Values are expressed as mean ±SD

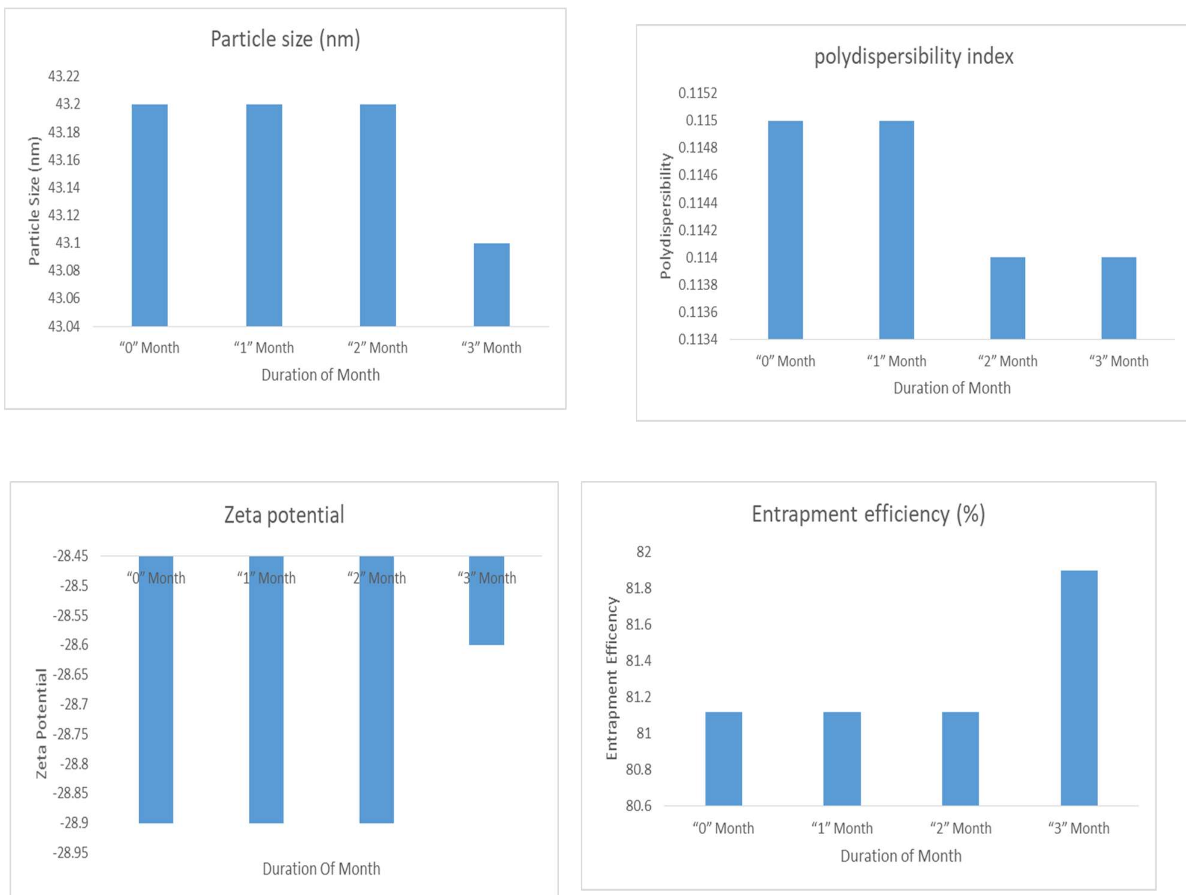


Fig 32 : Stability of Nanoparticles of F1

13. Preformulation study of Nanoparticle Tablet Dosage form :

i. Organoleptic studies

Powder of blend was found to be off-white.

Precompression parameters-:

1. Angle of Repose-ɵ

Table 24. Pre compression evaluation parameters- Angle of Repose (ɵ))

Batch	Sample weight (gm)	Height of pile (h) in cm	Radius (r) in cm			Angle of Repose (θ)			Mean θ	S.D (+-)	Angle of repose
			r1	r2	r3	θ1	θ2	θ3			
F1	5.005	1.4	2.6	2.7	2.8	27.93	27.90	28.80	28.20	±0.2	Excellent
F2	5.003	1.56	2.5	2.7	2.8	31.35	31.35	30.55	31.12	±0.0	Good
F3	5.002	1.5	2.8	2.6	2.7	27.90	26.55	26.55	27.05	±0.5	Excellent
F4	5.002	1.6	2.7	2.8	2.7	29.65	28.80	27.90	28.82	±0.4	Excellent
F5	5.001	1.5	2.6	2.8	2.6	29.65	31.36	29.65	30.23	±0.8	Excellent
F6	5.003	1.6	2.7	2.8	2.6	27.90	28.83	27.90	28.22	±0.4	Excellent
F7	5.005	1.5	2.8	2.6	2.5	29.35	29.69	31.34	29.80	±0.5	Excellent
F8	5.002	1.5	2.6	2.5	2.6	26.55	27.48	26.50	26.83	±0.6	Excellent
F9	5.003	1.4	2.5	2.5	2.6	26.30	26.61	26.73	26.55	±0.3	Excellent
F10	5.001	1.4	2.8	2.6	2.5	27.03	27.48	27.16	27.20	±0.4	Excellent
F11	5.002	1.6	2.6	2.6	2.8	27.44	27.19	27.45	27.30	±0.2	Excellent
F12	5.003	1.5	2.8	2.5	2.8	26.30	26.55	26.55	26.43	±0.2	Excellent

±S.D. n=3

2. Bulk density

Table 25. Pre compression evaluation parameters- Bulk density

Batch	Mass of powder M (gm)	Bulk volume of powder V_0			Bulk density Db			Mean Db	S.D. ±
		V_{01}	V_{02}	V_{03}	Db1	Db2	Db3		
F1	25.002	60	61	60	0.41	0.42	0.43	0.42	±0.001
F2	25.003	61	63	62	0.41	0.41	0.42	0.41	±0.002
F3	25.003	61	62	60	0.41	0.41	0.41	0.41	±0.005
F4	25.002	59	62	61	0.41	0.42	0.41	0.42	±0.004
F5	25.001	59	62	60	0.41	0.42	0.41	0.42	±0.001
F6	25.003	61	60	60	0.42	0.42	0.41	0.42	±0.005
F7	25.004	61	62	60	0.42	0.42	0.43	0.42	±0.005
F8	25.014	61	60	60	0.43	0.42	0.43	0.43	±0.005
F9	25.040	61	62	60	0.41	0.40	0.42	0.40	±0.005
F10	25.025	60	61	59	0.43	0.42	0.41	0.42	±0.005
F11	25.012	63	63	60	0.43	0.42	0.41	0.42	±0.005
F12	25.004	61	60	61	0.42	0.43	0.41	0.42	±0.005

±S.D. n=3

3. Tapped density (TD)

Table 26. Pre compression evaluation parameters- Tapped Density.

Batch	Mass of powder M (gm)	Bulk Volume	Tapped volume of powder V_t			Tapped density Dt			Mean Dt	S.D. ±
			V_{t1}	V_{t2}	V_{t3}	Dt1	Dt2	Dt3		
F1	25.001	63	58	57	59	0.40	0.42	0.44	0.42	±0.002
F2	25.002	63	57	59	58	0.40	0.40	0.40	0.40	±0.01

F3	25.004	63	59	57	58	0.42	0.44	0.43	0.43	±0.01
F4	25.002	63	57	59	58	0.44	0.40	0.42	0.42	±0.005
F5	25.003	63	59	58	57	0.41	0.40	0.42	0.41	±0.01
F6	25.004	63	58	59	57	0.44	0.42	0.46	0.44	±0.02
F7	25.002	63	58	57	59	0.41	0.42	0.43	0.42	±0.01
F8	25.004	63	57	59	58	0.41	0.42	0.41	0.41	±0.005
F9	25.002	63	58	57	59	0.46	0.42	0.41	0.43	±0.02
F10	25.004	63	59	57	58	0.41	0.40	0.40	0.40	±0.005
F11	25.002	63	57	59	58	0.40	0.44	0.48	0.44	±0.004
F12	25.003	63	57	58	59	0.41	0.41	0.43	0.43	±0.011

±S.D. n=3

4. Carr's Index

Table 27. Pre compression evaluation parameters- Carr's Index

Batch	Tapped Density (Dt)	Bulk Density (Db)	Carr's Index 100 x (Dt)-(Db)/ (Dt)	Flow Character
F1	0.43	0.42	2.33	Excellent
F2	0.43	0.41	238	Excellent
F3	0.43	0.42	2.33	Excellent
F4	0.43	0.42	2.33	Excellent
F5	0.44	0.42	2.33	Excellent
F6	0.43	0.42	2.33	Excellent
F7	0.43	0.42	2.33	Excellent
F8	0.43	0.42	2.33	Excellent
F9	0.42	0.41	2.38	Excellent
F10	0.43	0.42	2.33	Excellent
F11	0.43	0.42	2.33	Excellent
F12	0.43	0.42	2.33	Excellent

5. Hausner's ratio

Table 28. Pre compression evaluation parameters- Hausner's ratio

Batch	Tapped Density (Dt)	Bulk Density (Db)	Hausner Ratio	Flow Character
F1	0.44	0.43	1.02	Excellent
F2	0.43	0.41	1.04	Excellent
F3	0.43	0.40	1.07	Excellent
F4	0.43	0.42	1.02	Excellent
F5	0.42	0.42	1.00	Excellent
F6	0.44	0.42	1.04	Excellent
F7	0.43	0.42	1.02	Excellent
F8	0.43	0.41	1.04	Excellent
F9	0.42	0.42	1.00	Excellent
F10	0.41	0.41	1.00	Excellent
F11	0.43	0.42	1.02	Excellent
F12	0.43	0.41	1.04	Excellent

DISCUSSION

Physical properties such as bulk density, tapped density, percent compressibility index, Hausner ratio, angle of repose were determined for the prepared tablet blend. The tablet blend batches in which microcrystalline cellulose was used as diluent, the angle of repose is between 26.56° to 31.10°, this indicated the passable flow ability. This property may be attributed due to the presence of microcrystalline cellulose having filamentous particles as diluent. Also, the Carr's index and Hausner's ratio were found to be in the range of ≤ 18 and 1.02 to 4.76 respectively, indicating good flow and compressibility of the blends.

14. Post Compression Parameters

1. Evaluation of Tablets

Organoleptic properties

All batches (F1-F12) were assessed for organoleptic properties like color, odor, and taste and found to be acceptable in all aspect.

General appearance: The formulated tablets were assessed for its general appearance and observations were made for shape, colour and texture.

a. Shape- Round

b. Colour- off white

c. Texture- smooth

From the results obtained it was found that F1-F12 formulations has hardness, weight variation & friability within IP limit.

2. Weight Variation

Table 29. Weight variation test- F1-F12

Sr. No.	Parameter	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
1	**Weight variation (%)**	3.16	3.50	3.22	3.55	3.20	3.25	3.15	3.80	3.40	3.20	3.65	4.02

3. Thickness

Table 30. Tablet parameters (Batch F1-F12) - Thickness.

Batch	Thicknessin mm n=3				S.D. ±
	1	**2**	**3**	**Mean**	
F1	4.49	4.50	4.49	4.49	±0.005
F2	4.50	4.51	4.51	4.50	±0.005
F3	4.50	4.51	4.51	4.50	±0.005
F4	4.50	4.52	4.52	4.51	±0.005
F5	4.50	4.50	4.50	4.5	±0.011
F6	4.51	4.50	4.50	4.50	±0.005
F7	4.51	4.49	4.51	4.50	±0.000
F8	4.51	4.50	4.51	4.50	±0.005
F9	4.52	4.51	4.51	4.51	±0.005
F10	4.52	4.51	4.51	4.51	±0.005
F11	4.52	4.53	4.51	4.52	±0.005
F12	4.52	4.51	4.52	4.51	±0.005

±S.D. n=3

4. Hardness test: ±S.D. n=3

Table 31. Tablet parameters (Batch F1-F12) - Hardness test.

Batch	Hardness in kp n=3				S.D. ±
	1	**2**	**3**	**Mean**	
F1	5.3	5.4	5.6	5.4	±0.152753
F2	5.4	5.5	5.4	5.4	±0.057735
F3	5.6	5.5	5.4	5.5	±0.1
F4	5.4	5.5	5.5	5.4	±0.057735
F5	5.4	5.5	5.4	5.4	±0.057735
F6	5.6	5.6	5.5	5.5	±0.057735
F7	5.4	5.4	5.5	5.4	±0.057735
F8	5.4	5.5	5.5	5.4	±0.057735
F9	5.5	5.6	5.5	5.5	±0.057735
F10	5.6	5.5	5.5	5.5	±0.057735

F11	5.4	5.5	5.5	5.4	±0.057735
F12	5.6	5.5	5.5	5.5	±0.057735

5. Friability test

Table 32. Tablet parameters (Batch F1-F12) - Friability test.

Batch	weight of tablets before test (W1)	weight of tablets after test (W2)	Friability % %Friability = [(W1-W2)/W1] × 100
F1	255	243	0.125
F2	253	250	0.256
F3	251	249	0.356
F4	253	252	0.243
F5	250	247	0.469
F6	255	253	0.135
F7	256	255	0.124
F8	257	256	0.347
F9	250	247	0.467
F10	253	252	0.246
F11	250	246	0.244
F12	253	251	0.251

6. Disintegration time

Disintegration time for tablet F1-F12 was calculated. The data obtained is given below in the table.

Table 33. Tablet parameters (Batch F1-F12) - Disintegration time.

Batch	Disintegration Time (min)
F1	12.14
F2	13.450
F3	12.120
F4	13.42
F5	12.18
F6	11.45
F7	10.02
F8	12.04
F9	10.54
F10	10.48
F11	11.40
F12	11.06

7. Dissolution Study

Dissolution data of matrix tablets are reported in below respective tables. Dissolution study for each formulation was carried out in triplicate, in 7.4 P^H Phosphate Buffer. ±S.D. n=3

Table 34: Cumulative drug release (F1-F12)

Time(min)	F1	F2	F3	F4	F5	F6	F7
0	0	0	0	0	0	0	0
5	23.12±0.11	16.23±0.08	9.45±0.14	10.25±0.18	9.05±0.24	16.1±0.15	12.39±0.26
15	32.16±0.16	28.99±0.15	20.57±0.17	29.06±0.15	22.69±0.22	22.99±0.23	29.6±0.14
30	51.23±0.12	36.54±0.21	45.21±0.16	49.93±0.16	35.57±0.10	37.51±0.20	39.25±0.25
45	69.53±0.22	54.20±0.2	68.54±0.25	58.66±0.11	60.62±0.08	51.26±0.21	77.68±0.01
60	72.36±0.11	68.99±0.02	79.68±0.22	79.84±0.12	78.11±0.11	74.52±0.17	92.23±0.15

	F8	F9	F10	F11	F12
	0	0	0	0	0
	21.12±0.35	12.36±0.64	18.55±0.47	13.14±0.64	16.32±1.64
	34.15±0.47	33.12±1.04	38.74±1.53	35.78±1.57	27.88±0.83

	58.59±0.87	55.25±1.14	46.53±1.04	66.89±1.56	38.95±0.46
	65.62±1.04	77.15±0.58	72.56±1.05	78.66±0.53	62.15±0.46
	88.55±1.01	80.56±0.15	81.14±0.63	82.21±1.05	88.74±0.03

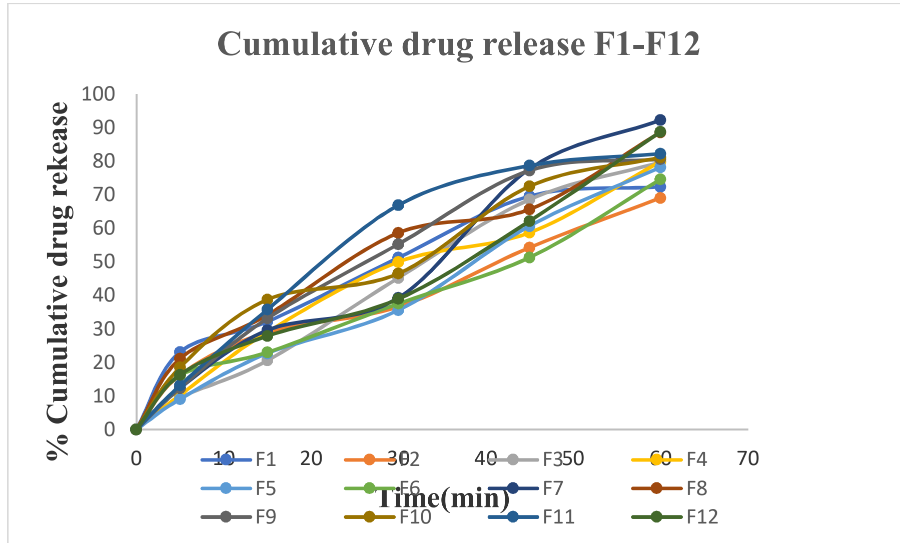


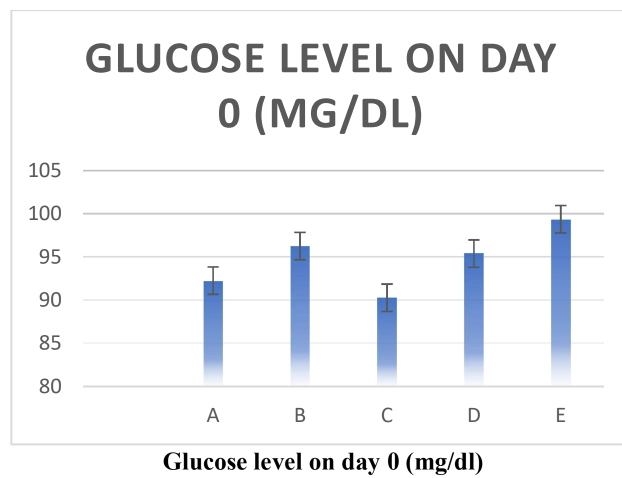
Figure 33: Cumulative drug release (F1-F12))

15. In vivo activity

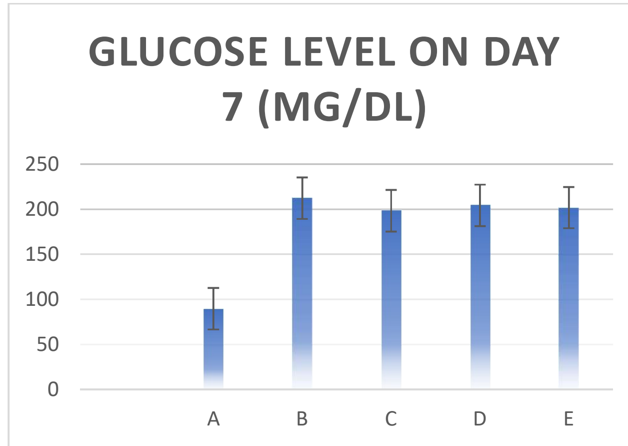
i. In vivo streptozotocin induced antidiabetic activity

Table 35: Streptozotocin induced antidiabetic activity

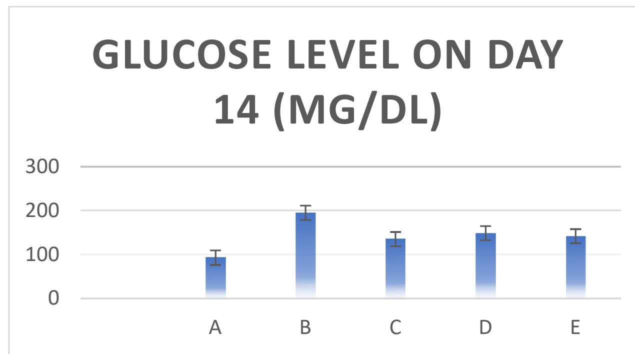
Sr. No	Groups	Glucose level on day 0 (mg/dl)	Glucose level on day 7 (mg/dl)	Glucose level on day 14 (mg/dl)	Glucose level on day 28 (mg/dl)
1	Positive control	92.23±3.68	89.56±4.38	92.59±2.56	91.47±1.96
2	Disease induces	96.24±3.4^{ns}	212.29±4.28$^{@}$	194.78±5.68$^{@}$	193.24±2.75$^{@}$
3	Glibencamide standard	90.25±7.63^{ns}	198.19±3.21^{ns}	134.69±3.64**	129.53±4.93**
4	EET	95.39±6.15^{ns}	204.23±8.32^{ns}	148.28±4.39**	137.47±2.38**
5	F1 isolated compound nanoparticles	99.34±8.25^{ns}	201.68±7.43^{ns}	141.38±5.96**	132.89±9.45**



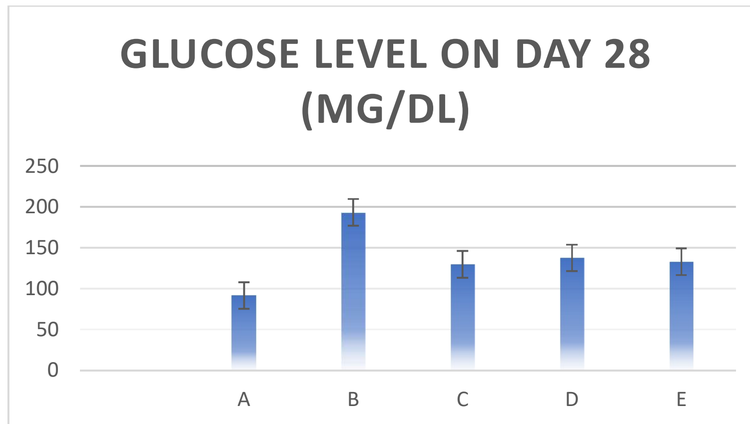
Glucose level on day 0 (mg/dl)



Glucose level on day 7 (mg/dl)



Glucose level on day 14 (mg/dl)



Glucose level on day 28 (mg/dl)

The results were expressed as mean SD (n=6), ns p>0.05,non- significant; $^{**}p<0.01$, When compared with positive control group. Based on the provide data on glucose levels over the course of the experiment, here is a conclusion drawn regarding the anti –diabetic activity.

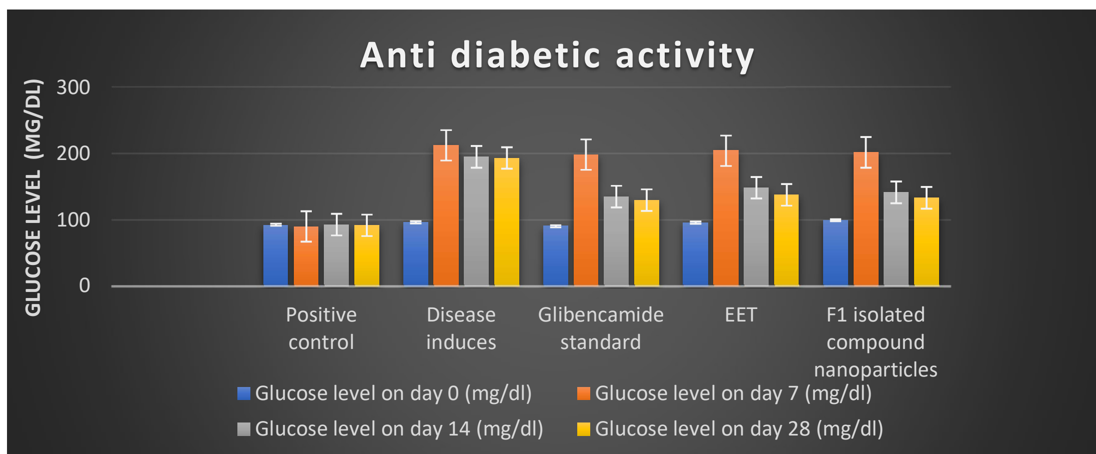


Figure 34: Streptozotocin induced antidiabetic activity of blood glucose

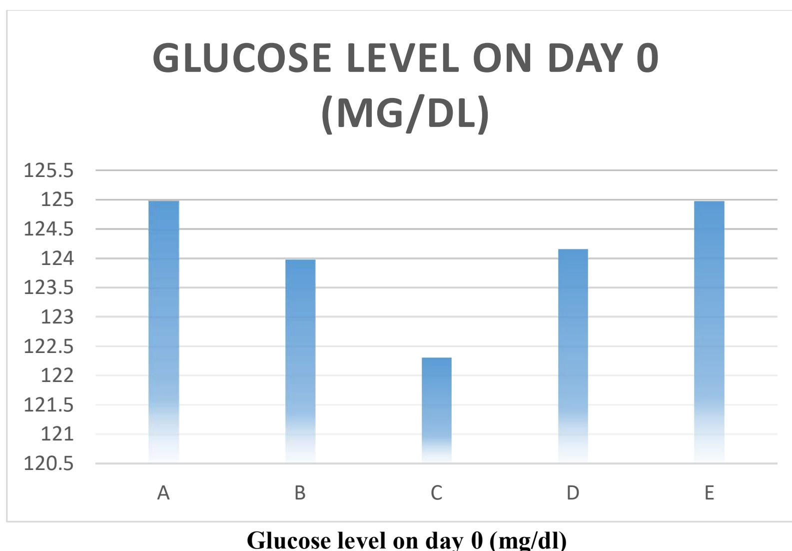
ii. Blood Glucose Lowering study (Hypoglycemic study):

Effect of Ethanolic Extract of EET Nanoparticle Formulation on Blood Glucose levels in Diabetic Wistar rat. The formulated nanoparticles of extract of Euphorbia thymifolia after administration in Swiss albino mice were studied for antidiabetic activity for 1 month.

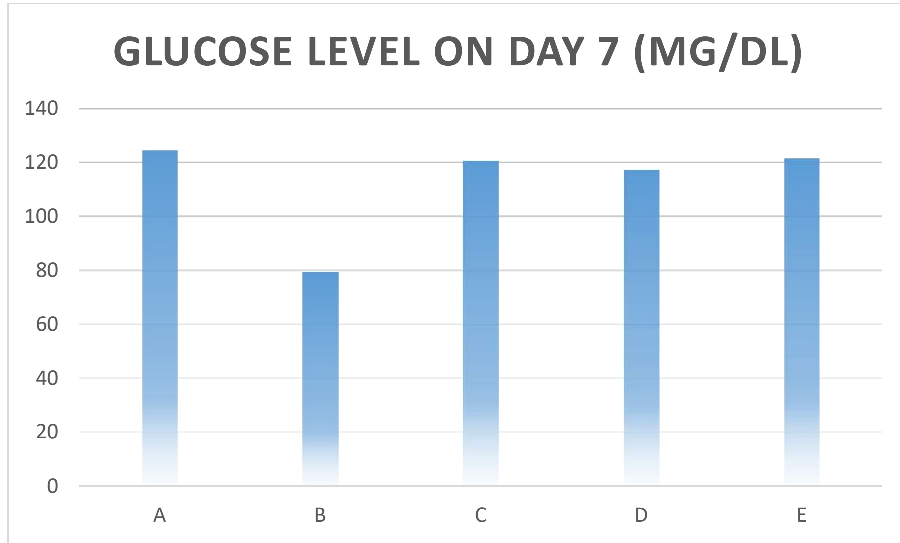
Table 36. Blood Glucose levels in Diabetic Wistar rat

Groups	**Glucose level on day 0 (mg/dl)**	**Glucose level on day 7 (mg/dl)**	**Glucose level on day 14 (mg/dl)**	**Glucose level on day 28 (mg/dl)**
Normal group	124.98±4.23	124.45±2.15	122.36±3.69	121.25±4.28
Diabetic control	123.97±1.36 ^{ns}	79.24±4.28$^{@}$	74.28±5.89$^{@}$	70.18±7.28$^{@}$
Standard	122.3±4.12 ^{ns}	120.57±2.13 ^{ns}	110.56±0.79**	108.68±0.98**
EET	124.16±3.85 ^{ns}	117.17±6.15 ^{ns}	112.85±2.85**	111.95±3.09**
F1 isolated compound nanoparticles	124.97±3.46 ^{ns}	121.58±2.36 ^{ns}	116.18±4.07**	110.68±5.09**

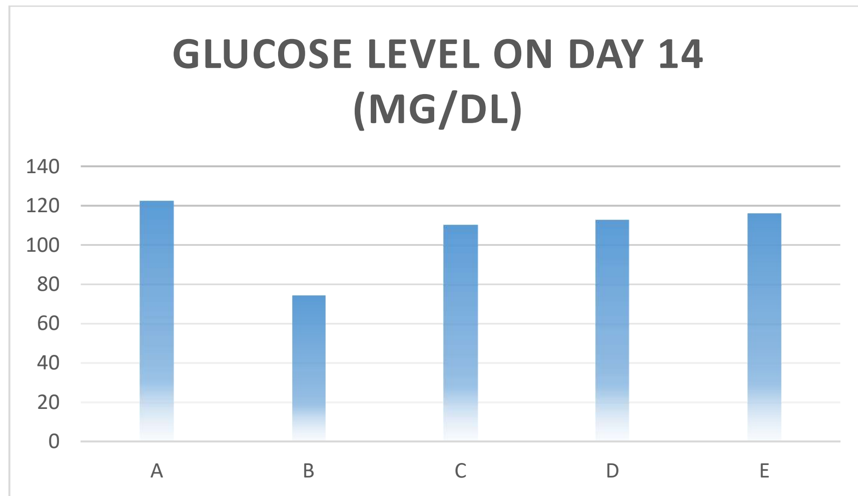
The results were expressed as mean SD (n=6), ns p>0.05, non- significant; $^{**}{}_{p<0.01}$, When compared with positive control group. Based on the provide data on glucose levels over the course of the experiment, here is a conclusion drawn regarding the anti –diabetic activity.



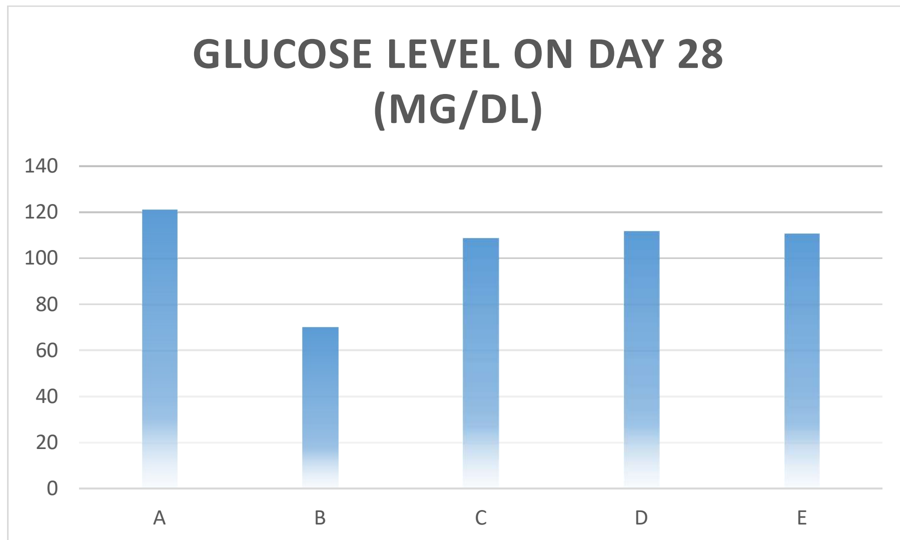
Glucose level on day 0 (mg/dl)



Glucose level on day 7 (mg/dl)



Glucose level on day 14 (mg/dl)



Glucose level on day 28 (mg/dl)

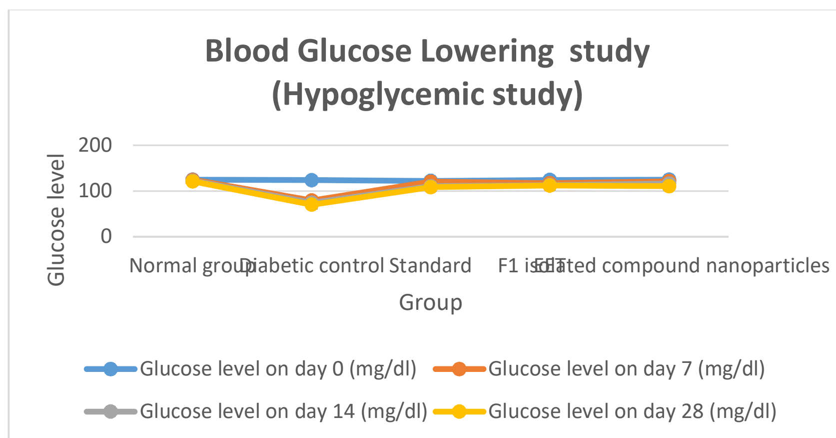


Figure 35: Blood Glucose levels in Diabetic Wistar rat)

16. CONCLUSION

- Euphorbia thymifolia was selected based on their literature claim for Antidiabetic & practical findings from their extractive yields.
- Present research work deals with formulation and evaluation of the Nanoparticles as a novel dosage form from extracts and isolated compound.
- Hence, these investigations provide strong support for the selected medicinal plants for this research work and which also ascertain its folk claims.
- The present research work helpful in development of efficacious nanoparticle in diabetes mellitus treatment.
- Also this research work may prove as an important tool for detection of possible mechanism of action of the herbal drugs.

Conflict Of Interest

The authors declare no conflict of interest.

REFERENCES

1. Kokate CK. Practical Pharmacognosy. Published by NiraliPrakashan New Delhi; 2004. p. 29-107.
2. Trease GE, Evans WC. Pharmacognosy. 12th Edition, ELBS Publications, London, 1985, 126-137.
3. Khandelwal, K_R. Practical pharmacognosy. Pragati Books Pvt. Ltd., 2008.
4. DeeptiSisodiya*, PragyaShrivastava Qualitative And Quantitative Estimation Of Bioactive Compounds Of Euphorbia Thymifolia L. Asian Journal of Pharmaceutical Education and Research Vol -6, Issue-3, July-September 2017.
5. Harborne, Jeffrey Barry. A guide to modern techniques of plant analysis. Chapman and Hall, 1973.
6. Wagner, Hildebert, and Sabine Bladt. Plant drug analysis: a thin layer chromatography atlas. Springer Science & Business Media, 1996.
7. Shrivastava, Rashmi, and Jyotsana Mishra. "Extraction, phytochemical screening, isolation and identification of bioactive compounds from extract of the plant Euphorbia Thymifolia Linn." Journal of Drug Delivery and Therapeutics 9, no. 3 (2019): 107-113.

8. Hussain, Riaz, Bakhat Ali, Muhammad Imran, and Abdul Malik. "Thymofolinoates A and B, new cinnamic acid derivatives from Euphorbia thymifolia." Natural product communications 7, no. 10 (2012): 1934578X1200701025.
9. Woldu, Minyahil A., and Jimma L. Lenjisa. "Nanoparticles and the new era in diabetes management." Int J Basic ClinPharmacol 3, no. 2 (2014): 277-84.
10. Hiroshi, Hikino, Kobayashi Masako, Suzuki Yutaka, and Konno Chohachi. "Mechanisms of hypoglycemic activity of aconitan A, a glycan from Aconitum carmichaeli roots." Journal of Ethnopharmacology 25, no. 3 (1989): 295-304.
11. Cohen, Margo Panush, and Van-Yu Wu. "[5] Purification of glycated hemoglobin." Methods in enzymology 231 (1994): 65-75.
12. Wolffenbuttel, Bruce HR, Donna Giordano, Henry W. Founds, and Richard Bucala. "Long-term assessment of glucose control by haemoglobin-AGE measurement." The Lancet 347, no. 9000 (1996): 513-515.
13. Baquer, Najma Zaheer, Dhananjay Gupta, and Jayadev Raju. "Regulation of metabolic pathways in liver and kidney during experimental diabetes: effects of antidiabetic compounds." Indian Journal of Clinical Biochemistry 13, no. 2 (1998): 63-80
14. Umrani, Rinku D., and Kishore M. Paknikar. "Zinc oxide nanoparticles show antidiabetic activity in streptozotocin-induced Type 1 and 2 diabetic rats." Nanomedicine 9, no. 1 (2014): 89-104.
15. Ranasinghe, Priyanga, ShehaniPigera, PriyadarshaniGalappatthy, Prasad Katulanda, and Godwin R. Constantine. "Zinc and diabetes mellitus: understanding molecular mechanisms and clinical implications." DARU Journal of Pharmaceutical Sciences 23, no. 1 (2015): 1-13.
16. Nasrollahzadeh, Mahmoud, MohaddesehSajjadi, S. Mohammad Sajadi, and Zahra Issaabadi. "Green nanotechnology." In Interface Science and Technology, vol. 28, pp. 145-198. Elsevier, 2019
17. Mamatha, G. C., E. Venkatanagaraju, K. Neelima, P. Madhulatha, and V. Madhuri. "Research Article Phytochemical Screening of Euphorbia thymifolia Linn." Phytochemical analysis 7: 7.
18. Parmar, Ghanshyam, and PundarikakshuduKilambi. "Comparative Pharmacognostic and Phytochemical Standardization of Euphorbia hirta L. and Euphorbia thymifolia L." (2017).
19. Ranasinghe, Priyanga, ShehaniPigera, PriyadarshaniGalappatthy, Prasad Katulanda, and Godwin R. Constantine. "Zinc and diabetes mellitus: understanding molecular mechanisms and clinical implications." DARU Journal of Pharmaceutical Sciences 23, no. 1 (2015): 1-13.
20. Nasrollahzadeh, Mahmoud, MohaddesehSajjadi, S. Mohammad Sajadi, and Zahra Issaabadi. "Green nanotechnology." In Interface Science and Technology, vol. 28, pp. 145-198. Elsevier, 2019.
21. Mamatha, G. C., E. Venkatanagaraju, K. Neelima, P. Madhulatha, and V. Madhuri. "Research Article Phytochemical Screening of Euphorbia thymifolia Linn." Phytochemical analysis 7: 7.
22. Nie, Xin, Zhejie Chen, Lan Pang, Lin Wang, Huajuan Jiang, Yi Chen, Zhen Zhang, Chaomei Fu, Bo Ren, and Jinming Zhang. "Oral Nano Drug Delivery Systems for the Treatment of Type 2 Diabetes Mellitus: An Available Administration Strategy for Antidiabetic Phytocompounds." International Journal of Nanomedicine 15 (2020): 10215.
23. Cavaiola, Tricia Santos, and Jeremy H. Pettus. "Management of type 2 diabetes: selecting amongst available pharmacological agents." Endotext [internet] (2017).
24. Vijay S. Patil, Kamalakar P. Nandre, Sougata Ghosh, Vaidya Jayathirtha Rao, Balu A. Chopade, Balasubramanian Sridhar, Sheshanath V. Bhosale, Sidhanath V. Bhosale; Synthesis, crystal structure and antidiabetic activity of substituted (E)- 3 -(Benzo [d]thiazol 2-ylamino) phenylprop2- en-1-one, European Journal of Medicinal Chemistry, 2013, 59, 304-309
25. Shrivastava, R. and Mishra, J., 2019. Extraction, phytochemical screening, isolation and identification of bioactive compounds from extract of the plant Euphorbia Thymifolia Linn. Journal of Drug Delivery and Therapeutics, 9(3), pp.107-113.
26. Ranasinghe, Priyanga, ShehaniPigera, PriyadarshaniGalappatthy, Prasad Katulanda, and Godwin R. Constantine. "Zinc and diabetes mellitus: understanding molecular mechanisms and clinical implications." DARU Journal of Pharmaceutical Sciences 23, no. 1 (2015): 1-13.
27. Nasrollahzadeh, Mahmoud, MohaddesehSajjadi, S. Mohammad Sajadi, and Zahra Issaabadi. "Green nanotechnology." In Interface Science and Technology, vol. 28, pp. 145-198. Elsevier, 2019
28. Mamatha, G. C., E. Venkatanagaraju, K. Neelima, P. Madhulatha, and V. Madhuri. "Research Article Phytochemical Screening of Euphorbia thymifolia Linn." Phytochemical analysis 7: 7.
29. Parmar, Ghanshyam, and PundarikakshuduKilambi. "Comparative Pharmacognostic and Phytochemical Standardization of Euphorbia hirta L. and Euphorbia thymifolia L." (2017).
30. Ranasinghe, Priyanga, ShehaniPigera, PriyadarshaniGalappatthy, Prasad Katulanda, and Godwin R. Constantine. "Zinc and diabetes mellitus: understanding molecular mechanisms and clinical implications." DARU Journal of Pharmaceutical Sciences 23, no. 1 (2015): 1-13.

31. Nasrollahzadeh, Mahmoud, MohaddesehSajjadi, S. Mohammad Sajadi, and Zahra Issaabadi. "Green nanotechnology." In Interface Science and Technology, vol. 28, pp. 145-198. Elsevier, 2019.
32. Pradeep Singh, Muhammad Arif, Abdul Qadir, PushpendraKannojia, Simultaneous Analytical Efficiency Evaluation Using an HPTLC Method for the Analysis of Syringic Acid and Vanillic Acid and Their Anti-Oxidant Capacity from Methanol Extract of Ricinuscommunis L. and Euphorbia hirta L, Journal of AOAC INTERNATIONAL, Volume 104, Issue 4, July-August 2021, Pages 1188–1195
33. Abou El-Soud, N., El-Laithy, N., El-Saeed, G., Wahby, M., Khalil, M., Morsy, F. and Shaffie, N., 2011. Antidiabetic activities of Foeniculumvulgare Mill. essential oil in streptozotocin-induced diabetic rats. Macedonian J Med Sci, 4(2), pp.139-146.
34. P. Stanely, Mainzen Prince; et al., Antidiabetic and antihyperlipidaemic effect of alcoholic Syzigiumcumini seeds in alloxan induced diabetic albino rats, Journal of Ethnopharmacology 91 (2004) 209–213.
35. Lokesh Deb, ChiranjibBhattacharjee, et al., Evaluation of Anti-Diabetic Potential of the Syzygium Cumini (Linn) Skeels By Reverse Pharmacological Approaches, Bulletin of Pharmaceutical Research 2013;3(3):135-45
36. Rao BK, Rao CH., Hypoglycemic and antihyperglycemic activity of Syzygium alternifolium (Wt.) Walp. seed extracts in normal and diabetic rats., Phytomedicine. 2001 Mar;8(2):88-93.
37. S Sadasivam, K Karthic; et al., Identification of α amylase inhibitors from Syzygium cumini Linn seeds, Indian Journal of Experimental Biology Vol 46, September 2008, pp. 677-680.
38. Saravanan G, Leelavinothan P., Effects of Syzygium Cumini Bark on Blood Glucose, Plasma Insulin and C-peptide in Streptozotocin induced Diabetic rats, Int J EndocrinolMetab 2006; 4: 96-105.
39. KalakotlaShanker et al,Antihyperglycemic and anti-hyperlipidemic effect of biologically synthesized silver nanoparticles and G. sylvestre extract on streptozotocin induced diabetic rats-an in vivo approach, Materials Letters 195 (2017) 240–244
40. Catherine Ulbricht et al,(2011) An Evidence-Based Systematic Review of Gymnema (Gymnema sylvestre R. Br.) by the Natural Standard Research CollaborationJournal of Dietary Supplements, 8(3):311–330, 2011
41. N. Supraja et al, (2017) Green Synthesis and Characterization of Silver Nanoparticles from Gymnema sylvestre Leaf Extract: Study of Antimicrobia ActivitiesInt.J.Curr.Microbiol.App.Sci(2017) 6(3): 530-540
42. M. Gomathi et al, (2020), Green synthesis of silver nanoparticles using Gymnema sylvestre leaf extract and evaluation of its antibacterial activity, South African Journal of Chemical Engineering 32 (2020) 1–4
43. Om Prakash Bangar, E.Edwin; et al., Antidiabetic activity of of a polyherbal formulation (Karnim Plus), International Journal of Green Pharmacy, July- September 2009, 211-214.
44. Margret Chandira, B.Jayakar, 2010 Formulation and evaluation of herbal tablets Containing Ipomoea Digitata Linn. Extract, International Journal of Pharmaceutical Sciences Review and Research, Volume 3, Issue 1, July–August.
45. H.S. Chandel, A. Pathak 2011Polyherbal formulations for antidiabetic therapy, International Journal of Pharmacy and Pharmaceutical Sciences, Vol 3 Suppl 3, , 226-228.
46. Mali PY, Panchal SS. 2013 A review on phyto-pharmacological potentials of Euphorbia thymifolia L. Ancient science of life. Jan;32(3):165.
47. Gupta, B., Srivastava, R. S., &Goyal, R.2007 Therapeutic uses of Euphorbia thymifolia: A review. Pharmacognosy Reviews, ,1,2,299-304.)