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## Extraction and biological evaluation of esterified lutein from marigold flower petals

**Saisugun J, Adi Lakshmi K, Gowthami Aishwarya K, Sneha Priya K, Sasidhar RLC, Suryanarayana Raju D and Venkateswara Rao B**

**Abstract**

*Tagetes erecta*, the Mexican marigold also called Aztec marigold is a species of genus *Tagetes*. *Tagetes erecta* is known for its high therapeutic values. These plants are rich in alkaloids, Terrene's, flavonoids, phenolic compounds etc. The dried and cleaned marigold flower petals were taken and lutein was extracted with hexane through conventional extraction by Soxhlet extractor. The esterified lutein was subjected to analytical procedures like TLC, UV-Visible Spectroscopy and IR Spectroscopy. The biological activities like Anti Diabetic Activity, Wound Healing Activity, *In-vitro* Coagulant Activity, Anti-inflammatory Activity was evaluated.

**Keywords:** *Tagetes erecta*, esterified lutein, anti-diabetic activity, wound healing activity

**Introduction**

*Tagetes erecta*, the Mexican marigold also called Aztec marigold is a species of genus *Tagetes* native to Mexico and Central America. Despite its being native to America, it is often called as African Mari gold. The common name in English marigold is derived from Mary's gold a name first applied to a similar plant native to Europe, *Calendula officinalis*. Some of the major *Tagetes* varieties are *Tagetes erecta* is also known as African marigold, *Tagetes patula* is also known as French marigold, *Tagetes tenuifolia* is also known as signet marigold.

*Tagetes* is a genus of annual or perennial, mostly herbaceous plants in the sunflower family (*Asteraceae*). It was described as a genus by Linnaeus in 1753. The name *Tagetes* is originated from the name of the Etruscan Tages. The most commonly cultivated varieties of *Tagetes* are known variously as African marigold, taxonomically known as *Tagetes erecta*. The genus *Tagetes* consists of 56 species.

African marigold are tall, erect growing plants upto 3 feet in height. The flowers are globe shaped and large, having both ray and disc florets. Flowers may measure upto 5 inches across. African marigolds are very good bedding plants. These flowers are yellow to orange and do not include red colored marigolds. The Africans marigold take longer to reach flowering stage than French marigold.

**Scientific classification**

Kingdom: *Plantae*

Order: *Asterales*

Family: *Asteraceae*

Subfamily: *Asteroideae*

Genus: *Tagetes*

Species: *T. erecta*

Binomial name: *Tagetes erecta*.

**Pharmacognostic, phytochemical and physicochemical parameters****Macroscopic characteristics**

*Tagetes erecta* flower has bright colour, aromatic odour and distinctly bitter taste. It has the length of 2-3cm and of thickness 3-5.5mm. Corolla is bright orange and calyx of dark green ovate type.

**Phytoconstituents**

Preliminary evaluation revealed that *Tagetes erecta* flowers contain phytoconstituents Such as tannins, phenolic compounds, flavonols, sterols, triterpenoids, saponins and alkaloids.

**Physicochemical parameters**

Loss on drying 7.46% w/w,  
 Total ash 4.95% w/w,  
 Acid insoluble ash 0.2% w/w,  
 Water soluble ash 1.65% w/w,  
 Sulphate ash 1.3% w/w,  
 Water soluble extractive value 72% w/w  
 Alcohol soluble extractive value 16.8% w/w.

**Pharmacological actions**

*Tagetes erecta* reported to have different pharmacological actions.

1. Anti-bacterial activity
2. Anti-microbial activity
3. Anti-oxidant activity
4. Hepato protective activity
5. Analgesic activity
6. Larvicidal activity
7. Insecticidal activity
8. Mosquitocidal activity
9. Nematicides activity
10. Wound healing activity
11. Anti-ulcer activity

**Uses**

*Tagetes erecta* is known for its high therapeutic values. These plants are rich in alkaloids, Terpenes, flavonoids, phenolic compounds etc. Since ancient era, all the parts of the plants are used in medicine for curing many diseases. Leaves are used as an antiseptic agent and also used in kidney troubles, muscular pain, and piles and applied to boil.

The flowers are used to cure fever, epileptic fits according to ayurveda, astringent, carminative and stomachic, scabies and liver complaints and is also employed in diseases of the eyes. They are also used to purify blood and flower juice is given as remedy for bleeding piles and is also used in rheumatism and bronchitis.

In Brazil flower and leaf infusion used as vermifuge. Mexicans used decoctions of flowers and leaves as diuretics and carminative. Other uses include it is used for anaemia, amenorrhoea, abdominal pain, muscular and bone pain. Internally used for indigestion, cough and dysentery. Externally used for ulcers, eczema, sore eyes.

Flower extract was found to contain biologically useful lutein compounds and studied for use as nutritional supplement and as poultry food colorant. The petals yield a natural dye, the colorants consisting mainly of carotenoid - lutein and flavonoid with crude extracts used for dyeing textiles.

The florets of *Tagetes erecta* are rich in the Orange-yellow carotenoid lutein and are used as a food colour in the European Union for food.

**Chemical constituents**

Lutein from *Tagetes erecta* is produced from marigold oleoresin. The marigold oleoresin is extracted from dried marigold flower petals with hexane and contains lutein, lutein esters, other carotenoids and waxes. Purified lutein is obtained from the oleoresin by saponification and crystallisation.

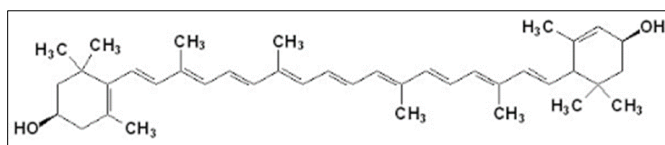
Chemical Abstract Service (C.A.S) number : 127-40-2

Chemical formula :  $C_{40}H_{56}O_2$

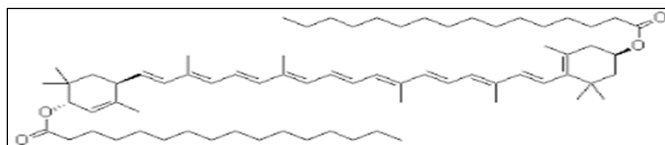
Molecular weight : 568.88

Lutein is a free flowing orange red powder. It is insoluble in water, soluble in hexane. Lutein is intended for use as a colouring agent and a nutrient supplement.

Lutein is an oxycarotenoid or xanthophyll, containing two cyclic end groups and basic C-40 isoprenoid structure common to all carotenoids. It is one of the major constituents and the main pigment of *Tagetes erecta*.



**Fig 1:** Chemical structure of lutein



**Fig 2:** Chemical structure of esterified lutein

It occurs in many kinds of fruits and vegetables, especially in leafy vegetables, but also in the yolk and eye tissues. Lutein acts as an effective antioxidant, namely in the protection of eyes, because it neutralizes free radicals formed by the action of ultraviolet radiation on eye retina. Humans are not able to synthesise lutein, so they can acquire it solely by the consumption of fruits, vegetables. In plants lutein is present either in the form of free lutein in leafy vegetables such as spinach, cabbage and broccoli, or in the form of esters with fatty acids in fruits and vegetables like mango, orange, papaya, red or green pepper, yellow corn etc. The content of lutein in natural sources depends on their kind, variety, level of maturity, part of fruit, and also on the way of processing by heat, preservation. Marigold flower (*Tagetes erecta*) represents a rich source of lutein. It is grown for business purposes in Mexico, Peru, Ecuador, Spain, India, and China.

**Materials and Methods****Extraction procedure****Plant source**

The Marigold flowers (*Tagetes erecta*) were brought from Rythu bazaar, Behind RTC bus stand, Guntur. They were authenticated by Pharmacognosy department by Smt. P. Vijetha M. Pharm. The flowers are cleaned and the petals were removed from the flowers and the petals were dried under sunlight such that water content would be removed from them.

**Chemicals used**

Hexane

Purified Water

**Equipment used**

Soxhlet extractor

Simple distillation apparatus

**Extraction process**

Conventional extraction of lutein ester from Marigold flower petals was performed in soxlet extractor composed of a 500ml round bottom flask, extractor and condenser. The extractor was filled with plant material. Extraction solvent used for the isolation of lutein ester was hexane. Volume of the extraction solvent used per 25gm of raw material was equal to 250ml. Extraction was stopped when saturated solution was obtained.

**Analytical procedures****Thin layer chromatography**

**Mobile phase used:** Diethyl ether, Methanol (Ratio – 3:1)

**Solvent used:** Hexane.

**Procedure**

**Dimensions of the plate:** 4 × 2.5 cm.

Initially the TLC chamber was saturated with the mobile phase. The Solvent was taken into the ignition tube and the sample was transferred into ignition tube using capillary tube and it was mixed thoroughly until the sample gets dissolved in the solvent. The TLC plate was marked 2cm above the base by using pencil and scale. The sample was spotted on TLC plate and observed under the UV chamber for recognizing the spot. Now the TLC plate was placed in TLC chamber containing mobile phase. The solvent starts eluting from the base and the plate was removed from the TLC chamber when the mobile phase elutes upto 3/4<sup>th</sup> of the TLC plate. The TLC plate was placed in the UV chamber for recognizing the eluted sample.



**Fig 3:** TLC Plate

$$RF = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}} = \frac{2.5}{3.4} = 0.73$$

**UV-visible spectroscopy**

**Preparation of the test sample:** The unknown amount of sample was taken and it was dissolved in hexane.

**Procedure:** Initially the equipment UV – VISIBLE Spectrophotometer (LAB INDIA UV-3000<sup>+</sup>) was runned by placing the solvent hexane in both reference and test cuvettes and the system was made auto zero by arranging the wave length range 420 - 460nm.

Now, the test sample (Esterified lutein) was taken into the test cuvette and the equipment was runned by placing the wavelength range 420 - 460nm.

The abs option maximum ( $\lambda$  max) of esterified lutein was found to be 442nm.

**IR spectroscopy**

We used BRUKER ALPHA F.T.IR instrument and identified different functional groups by observing their respective wave numbers (cm<sup>-1</sup>).

**Table 1:** Ranges of FTIR

Wave Number (cm <sup>-1</sup> )	Functional Group
3000-2950	Alkanes (stretch)
2900-2800	Aldehydes
3000-2850	Alkanes
1740-1720	Aldehydes
1725-1705	Ketones
1725-1700	Carboxylic acid
1680-1630	Amides
1730-1750	Ester
1680-1600	Alkene
1550-1350	Nitro
1350-1000	Amine
1350-1140	Sulphones, Sulphonyl chloride, Sulfates, Sulphonamides
1400-1000	Flourides
1300-1000	Alcohol, Ether, Ester, Carboxylic acids
785-540	Chloride

**Pharmacological screening****Animals used**

- Species/Common name : Albino wistar rats
- Age/weight/size : 150-250 g
- Gender : Either sex
- Number : 28
- Source of animals : Mahavir Enterprises, Hyderabad.

Healthy albino wister rats of either sex, housed in animal house of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chandra Mouli Puram, Chowdavaram, Guntur were selected and maintained under standard laboratory conditions of light at 23±2 °C and 55±5% R.H. The animals housing and handling were done in accordance with CPCSEA guidelines. The experiments were conducted as per the norms of Institutional Animal Ethics Committee (IAEC). The animals were given standard rat pellet feed and tap water. After one week of acclimatization, rats were randomly selected and grouped into different groups.

**Substances used**

- Esterified lutein from *Tagetes erecta* flower petals (ELTFP)
- Alloxan monohydrate
- Hexane
- Dimethyl Sulphoxide (DMSO)
- Safromycin ointment
- Phosphate buffer saline (PBS)
- Methanol
- Gelatin sponge
- EDTA
- Carrageenan
- Tween 80
- Hydroxyl Propyl Methyl Cellulose(HPMCE<sub>5</sub>)
- Eggs

**Equipments**

- Glucometer( Dr. Morpen)
- Animal weighing balance
- Incubator (Elite scientific)
- Centrifuge (REM I)
- Plethysmograph (MKM Chennai)

**Apparatus required**

- Beaker
- Oral feeding needle

- c. Pipette
- d. Volumetric flask
- e. Petridishes
- f. Syringes
- g. Lancets

### Anti-Diabetic Activity

#### Induction of Experimental Diabetes

##### Alloxan Induced Diabetes

Rats were induced diabetes by the administration of simple intraperitoneal dose of alloxan monohydrate (150mg/kg). Two days after alloxan introduction, rats were screened for blood glucose levels. All animals were allowed for free access to water and pellet diet and maintained at room temperature in respective cages.

#### Preparation of test sample

The 40mg/kg, 80mg/kg esterified lutein from *Tagetes erecta* flower petals (ELTFP) was dissolved individually in 0.5ml of hexane and then it was dissolved in the 2ml of Dimethyl Sulphoxide (DMSO).

#### Experimental Design

In present investigation, a total of 24 rats (18 diabetic surviving rats & 6 normal rats) were taken and divided into 4 groups of 6 rats each

**Group-I:** Normal untreated rats

**Group-II:** Alloxan Induced Rats (150mg/kg)

**Group-III:** Alloxan + esterified lutein from *Tagetes erecta* flower petals (ELTFP) 40mg/kg PO.

**Group-IV:** Alloxan + esterified lutein from *Tagetes erecta* flower petals (ELTFP) 80mg/kg PO.

#### Biochemical Analysis

The animals were anaesthetized and tail punctured, blood was collected and glucose levels were measured by using glucometer for every 24hrs for 3 days. Effect of esterified lutein from *Tagetes erecta* flower petals (ELTFP) on the body weight and fasting blood glucose in normal & diabetic rats was noted.

#### Wound Healing Activity

Chick Chorioallantonic membrane (CAM) model. In this study angiogenesis activity of esterified lutein from *Tagetes erecta* flower petals (ELTFP) was analysed by this method. The test sample esterified lutein from *Tagetes erecta* flower petals (ELTFP) was stored in air tight glass container at 4 °C light controlled environment. 50mg, 100mg test sample was prepared in phosphate buffer saline (PBS) were sterilized by passing through a syringe filter (0.22µm). Safromycin ointment (50µg) was prepared (std) in sterile PBS. Hen eggs were procured from hatchery and were cleaned and decontaminated using alcohol. For nine days they were incubated at 37 °C in incubator. A small window of 1.0cm<sup>2</sup> is made on the shell of egg and opened, Gelatin sponges were cut into approximately 2mm<sup>3</sup> pieces and loaded with 50mg of test sample, 100mg of test sample and 50mg of standard sample (Safromycin ointment) and placed at the junction of 3 different eggs, after that the window is closed with tape. One egg was kept as control. The sealed eggs were incubated at 37 °C in a well humidified chamber for 9 days. Then the eggs are opened and new blood vessel formation was observed in CAM containing sponge with esterified lutein from *Tagetes erecta* flower petals (ELTFP) which are compared with CAM containing sponge with standard.

### In-vitro Coagulant Activity

#### Preparation of the test sample

20mg of esterified lutein from *Tagetes erecta* flower petals (ELTFP) was dissolved in 1 ml of hexane.

#### Procedure

Initially 3 centrifuge tubes were taken and their weights were noted individually. Fresh blood was taken by retro orbital puncturing of the rat. The 2.5ml blood was filled in the 3 centrifuge tubes. The EDTA was added to the 2 centrifuge tubes to prevent the clotting and to one of the EDTA added centrifuge tube esterified lutein from *Tagetes erecta* flower petals (ELTFP) was added. The centrifuge tube with only Blood was marked as (A), the centrifuge tube with EDTA and Blood was marked as (B), the centrifuge tube with EDTA, Blood and ELTFP was marked as (C).

Place the centrifuge tubes in the centrifuge and operated at 3000rpm for about 10 mins. Then remove the plasma carefully without disturbing the serum. Then the weight of the centrifuge tubes were noted and the extent of clot formed in the 3 centrifuge tube were observed.

#### Anti-inflammatory activity

The anti-inflammatory activity of the esterified lutein from *Tagetes erecta* flower petals (ELTFP) was determined by the acute paw oedema method in rats.

The inflammatory reaction is readily produced in rats in the form of paw oedema with the help of irritants. The carrageenan (1%) was injected to the dorsum of the foot of rats and it produces acute paw oedema within few minutes of the injection. Carrageenan-induced paw oedema is the most commonly used method in experimental pharmacology. Carrageenan is a sulphated polysaccharide obtained from seaweed (Rhodophyceae) and by causing the release of histamine, 5-HT, bradykinin and prostaglandins-I produces inflammation and oedema.

#### Preparation of the carrageenan solution

1 gram carrageenan was weighed accurately and it was dissolved in the 100ml of purified water (1%).

#### Preparation of the test sample

500mg/kg body weight esterified lutein from *Tagetes erecta* flower petals (ELTFP) was weighed and it was made into the suspension by using the Hydroxyl Propyl Methyl Cellulose (HPMCE<sub>5</sub>) by adding little bit of tween80.

#### Equipment

Plethysmograph it is a simple apparatus containing mercury. The mercury displacement due to dipping of the paw can be directly read from scale attached to the mercury column (or) adjusting the mercury level in the arm B to the original level by moving arm B up/down and noting the volume required to bring the level in both the arms equal.

#### Procedure

4 rats were taken and made a mark on the hind paws (right & left) just beyond tibio-tarsal junction, so that every time the paw is dipped in the mercury column up to the fixed mark to ensure contact paw volume.

Note the initial paw volume (both right & left) of each rat by mercury displacement method.

To all the 4 rats the right paw was taken as reference non-inflamed paw for comparison. One rat is was kept as the control (Group-I).

There left paw volumes are noted for the 3 rats. And after 30mins the carrageenan solution was injected to the left paw of the 3 rats.

One rat is kept as the toxic control (Group-II), one rat is kept as the standard (Group-III) and one rat is kept as the test (Group-IV).

After 15min, to the rat (Group-III) diclofenac sodium(standard) was injected 5mg/kg body weight was injected and to the rat (Group-IV) esterfied lutein from *Tagetes erecta* flower petals (ELTFP) suspension was given through oral route.

Paw volumes (both right & left) of each rat are noted for every 15 minutes and then the mean difference in the volume of the right and left paw was observed.

**Results and Discussion**

**Anti-iabetic activity**

The present investigation indicate that esterfied lutein from *Tagetes erecta* flower petals (ELTFP) shows significant anti-diabetic activity in rats. In the present study alloxan induced rats showed significant increased in body weight. Administration of esterfied lutein from *Tagetes erecta* flower petals (ELTFP) 40mg/kg and 80mg/kg decreased the body weight within 3 DAYS. Fasting blood glucose levels of diabetic controlled rats was higher than those of normal rate was higher than those of normal rats. A significant dose dependent decrease in blood glucose levels were observed in diabetic treated group from an initial level of 180mg/dl to the level of 149mg/dl and from 180mg/dl to 92mg/dl after the treatment at a dose of 40mg/kg and 80mg/kg respectively for 3 days.

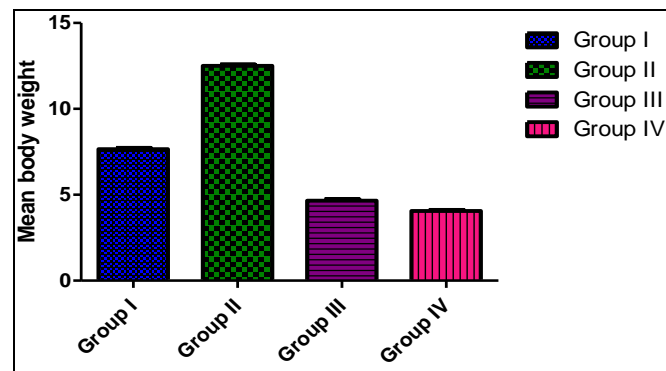
**Result on body weight**

**Table 2:** Result on body weight of rat

S. No	Group*	Initial body weight (gms)	Final body weight (gms)	Mean weight gain(+)/loss(-)
1.	I	198.5	206.16	(+7.66)
2.	II	204.16	198.65	(-)12.51
3.	III	189.65	194.31	(+)4.66
4.	IV	194.50	198.56	(+)4.06

The mean deviation <0.05

\*Group-I: Normal untreated rats, Group-II: Diabetic Control rats, Group-III: Diabetic rats given esterfied lutein from *Tagetes erecta* flower petals (ELTFP) 40mg/kg orally, Group-IV: Diabetic rats given esterfied lutein from *Tagetes erecta* flower petals (ELTFP) 80mg/kg body weight orally



The mean deviation <0.05

Effect of Lutein on body weight

Group-I: Normal untreated rats, Group-II: Diabetic Control rats, Group-III: Diabetic rats given esterfied lutein from *Tagetes erecta* flower petals (ELTFP) 40mg/kg body weight orally, Group-IV: Diabetic rats given esterfied lutein from *Tagetes erecta* flower petals (ELTFP) 80mg/kg body weight orally

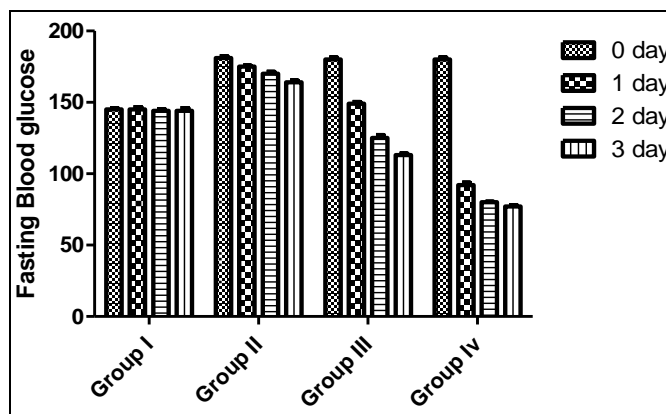
**Graphical representation 3:** Effect of Esrerified Lutein on body weight of diabetic rats

**Table 3:** Result on fasting blood glucose levels

S. No	Group*	Fasting blood glucose levels(mg/dl)			
		0 days	1 day	2 days	3 days
1.	I	145	145	144	144
2.	II	181	175	170	164
3.	III	180	149	125	113
4.	IV	180	92	80	77

The mean deviation for 0 days = 171.5±8.836, for 1 day = 140.3±17.40, for 2 days = 170±18.93, for 3 days = 124.5±18.99

\*Group-I :- Normal untreated rats, Group-II :- Diabetic Control rats, Group-III :- Diabetic rats given esterfied lutein from *Tagetes erecta* flower petals (ELTFP) 40mg/kg body weight orally, Group-IV Diabetic rats given esterfied lutein from *Tagetes serecta* flower petals (ELTFP) 80mg/kg body weight orally .



The mean deviation for 0 days = 171.5±8.836, for 1 day = 140.3±17.40, for 2 days = 170±18.93, for 3 days = 124.5±18.99

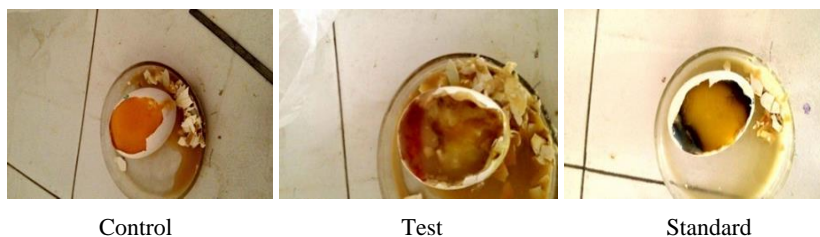
Effect of Lutein on Fasting blood glucose

Group-I: Normal untreated rats, Group-II: Diabetic Control rats, Group-III: Diabetic rats given esterfied lutein from *Tagetes erecta* flower petals (ELTFP) 40mg/kg body weight orally, Group-IV: Diabetic rats given esterfied lutein from *Tagetes erecta* flower petals (ELTFP) 80mg/kg body weight orally .

**Graphical representation**

4 Effect of Esterfied Lutein on fasting blood glucose levels of diabetic rats

**Wound Healing Activity**



**Fig 4(A, B, C):** Effect of Esterified lutein on Angiogenesis

The Test Sample is moderately angiogenetic when compared to the standard.

\*The centrifuge tube with only Blood was marked as (A), the centrifuge tube with EDTA and Blood was marked as (B), the centrifuge tube with EDTA, Blood and ELTFP was marked as (C).

**In-vitro coagulant activity**



EDTA + Blood



EDTA+Blood+ELTFP

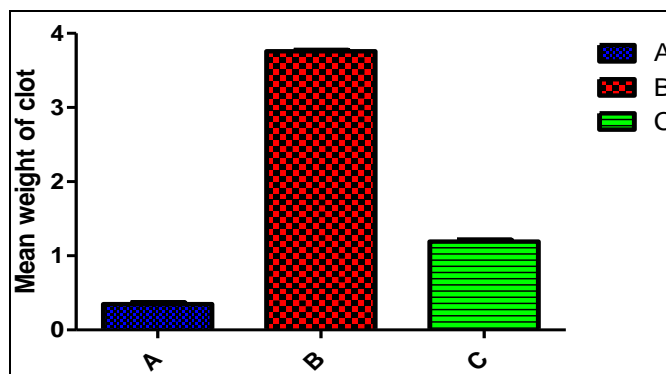


Blood

**Fig 5:** Effect of Esterified lutein on blood coagulant activity

**Table 4:** Result of blood coagulant activity

S. No	Group*	Initial weight (gms)	Final weight (gms)	Mean weight loss
1.	A	14.10	13.75	0.35±0.18
2.	B	14.78	11.02	3.76±0.0014
3.	C	15.10	13.87	1.23±0.02



Effect of Lutein on weight of clot

The centrifuge tube with only Blood was marked as (A), the centrifuge tube with EDTA and Blood was marked as (B), the centrifuge tube with EDTA, Blood and ELTFP was marked as (C).

**Graphical representation 5:** Effect of Esterified Lutein on weight of clot

**Anti-inflammatory Activity**

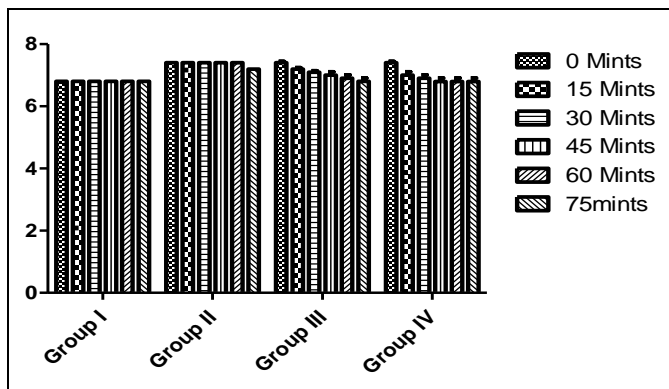
**Table 5:** Result of Anti-Inflammatory Activity

S. No	Group	Paw volume (ml) measured by mercury displacement											
		0min		15min		30min		45min		60min		90min	
		R*	L*	R	L	R	L	R	L	R	L	R	L
1.	Group-I	6.7	6.8	6.7	6.8	6.7	6.8	6.7	6.8	6.7	6.8	6.7	6.8
2.	Group-II	6.7	7.4	6.7	7.4	6.7	7.4	6.7	7.4	6.7	7.4	6.7	7.2
3.	Group-III	6.7	7.4	6.7	7.0	6.7	6.9	6.7	6.8	6.7	6.8	6.7	6.8
4.	Group-IV	6.7	7.4	6.7	7.2	6.7	7.1	6.7	7.0	6.7	6.9	6.7	6.8

The standard deviation for 0 min = 7.25±0.1500, for 15 min = 7.10±0.1291, for 30 min = 7.050±0.1323, for 45 min = 7.00±0.144, for 60 min = 6.975±0.1436, for 75 min = 6.900±0.100

Group-I -Control rat, Group-II - Rat given with carrageenan solution (toxic control), Group-III - Rat given with diclofenac sodium (standard), Group-IV - Rat given with esterified lutein from *Tagetes erecta* flower petals (ELTFP) suspension.

\*R- Right Paw (reference) and L-Left Paw.



Effect Lutein on Inflammation

The standard deviation for 0 min =  $7.25 \pm 0.1500$ , for 15 min =  $7.10 \pm 0.1291$ , for 30 min =  $7.050 \pm 0.1323$ , for 45 min =  $7.00 \pm 0.144$ , for 60 min =  $6.975 \pm 0.1436$ , for 75 min =  $6.900 \pm 0.100$

Group-I -Control rat, Group-II - Rat given with carrageenan solution (toxic control), Group-III - Rat given with diclofenac sodium (standard), Group-IV - Rat given with esterified lutein from *Tagetes erecta* flower petals (ELTFP) suspension.

**Graphical representation 6:** Effect of Esterified Lutein on Inflammation

### Conclusion

Research is a never ending process where the new things will be discovered based on the available proofs and from past work. In our current study we have worked on the esterified lutein extracted from the marigold flower petals and we are concluding that it has good Anti-Diabetic activity with excellent results. Esterified lutein also shows acceptable activities like wound healing, Coagulant and Anti-inflammatory. We are planning our Anti-diabetic activity of esterified lutein for further investigation.

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