International Journal of Chemical Science
Online ISSN: 2523-2843, Print ISSN: 2523-6075
www.chemicaljournals.com
Volume 1; Issue 1; September 2017; Page No. 14-22

Extraction and biological evaluation of esterfied lutein from marigold flower petals

[1] Saisugun J, [2] Adi Lakshmi K, [3] Gowthami Aishwarya K, [4] Sneha Priya K, [5] Sasidhar RLC, [6] Suryanarayana Raju D, [7] Nagaraju B, [8] Venkateswara Rao B

[1, 2, 3, 4] B. Pharmacy Student Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chandramoulipuram, Chowdavaram, Guntur, Andhra Pradesh, India

[5] Asoociate Professor, Department of Pharmaceutics Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chandramoulipuram, Chowdavaram, Guntur, Andhra Pradesh, India

[6] Lecturer, Department of Pharamaceutical Chemistary Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chandramoulipuram, Chowdavaram, Guntur, Andhra Pradesh, India

[7] Lecturer department of Pharamacology Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chandramoulipuram, Chowdavaram, Guntur, Andhra Pradesh, India

[8] Assistant Professor, Department of Pharamaceutical Analysis Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chandramoulipuram, Chowdavaram, Guntur, Andhra Pradesh, India

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, Terpenes, flavonoids, phenolic compounds etc. The dried and cleanes marigold flower petels were taken and lutein was extracted with hexane through conventional extraction by soxlet extractor. The esterfied lutein was subjected to analytical procedures like TLC, UV-Visible Spectroscopy and IR Spectroscopy. The biologiacal activities like Anti Diabetic Activity, Wound Healing Activity, *In-vitro* Coagulant Activity, Anti-inflammatory Activity was evaluated.

Keywords: *Tagetes erecta*; esterfied lutein; Anti Diabetic Activity; Wound Healing Activity; *In-vitro* Coagulant Activity ; Anti-inflammatory 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 Marigold. 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 *Tagets erecta* flowers contain phytoconstituents such as tannins, phenolic compounds, flavonols, sterols, triterpinoids, 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, Sulphated ash1.3%w/w,

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,
Sulphated ash1.3%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. Nematicidal 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, 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 Absract 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.*

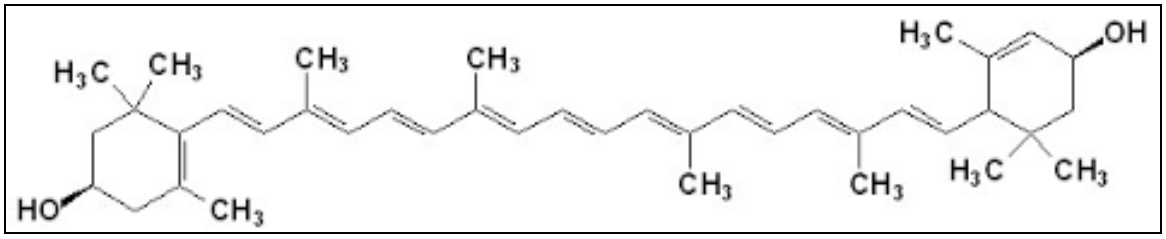


Fig 1: Chemical Strucuture of Lutein

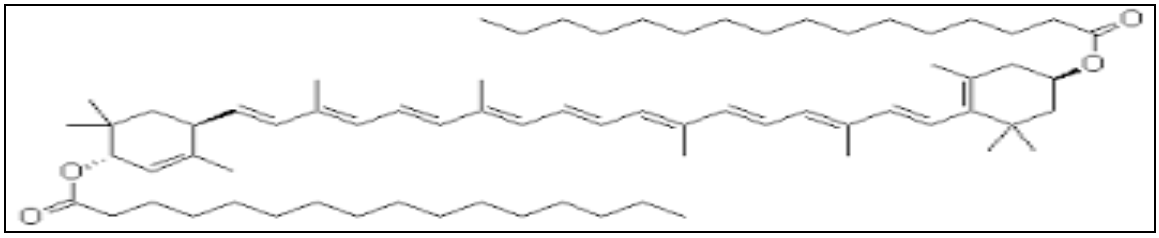


Fig 2: Chemical Strucuture of Esterified Lutein

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 neutralises 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, China.

Materials & Methods

Extraction Procedure

Plant Source: The Marigold flowers (*Tagetes erecta*) were brought from Rythu bazaar, Behind RTC bus stand, Guntur. They were aunthenticated by Pharmacognosy department by Smt. P. Viijetha 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

a. Hexane
b. Purified Water

Equipment Used

a. Soxhelet extractor
b. 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 satured 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 3/4th of the TLC plate. The TLC plate was placed in the UV chamber for recognizing the eluted sample.

Fig 3 TLC PLATE

R_f = Distance travelled by solute = 2.5 = 0.73
Distance travelled by solvent front 3.4

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+) 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 absoption maximum (λ 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-1).

Table 2: Ranges of FTIR

Wave Number (cm-1)	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

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

Healthy albino wister rats of either sex, housed in animal house of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences,Chandramoulipuram,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

a. Esterfied lutein from Tagetes erecta flower petals (ELTFP)
b. Alloxan monohydrate
c. Hexane
d. Dimethyl Sulphoxide (DMSO)
e. Safromycin ointment
f. Phosphate buffer saline (PBS)
g. Methanol
h. Gelatin sponge
i. EDTA
j. Carrageenan
k. Tween 80
l. Hydroxyl Propyl Methyl Cellulose (HPMCE5)
m. Eggs

Equipments

a. Glucometer (Dr Morpen)
b. Animal weighing balance
c. Incubator (Elite scientific)
d. Centrifuge (REM I)
e. Plethysmograph (MKM Chennai)

Apparatus Required

a. Beaker
b. Oral feeding needle
c. Pipette
d. Volumetric flask
e. Petridishes
f. Syringes
g. Lancets

Anti Diabetic Activity

Induction of Experimental Diabetes: Alloxan Indused 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 esterfied 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 rates each

Group I:- Normal untreated rats
Group II:- Alloxan Induced Rats (150mg/kg)
Group III:- Alloxan + esterfied lutein from *Tagetes erecta* flower petals (ELTFP) 40mg/kg PO.
Group IV:- Alloxan + esterfied 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 esterfied 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 esterfied lutein from *Tagetes erecta* flower petals (ELTFP) was analysed by this method. The test sample esterfied 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 1.0cm² is made on the shell of egg and opened, Gelatin sponges were cut into approximately 2mm³ 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 esterfied lutein from *Tagetes erecta* flower petals (ELTFP) which are compared with CAM containing sponge with standard.

3. *In-vitro* Coagulant Activity

- Preparation of the test sample

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

- Procedure

Initially 3 centrifuge tubes were taken and there 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 esterfied 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.

4. Anti-inflammatory Activity

The anti-inflammatory activity of the esterfied lutein from Tagetes erecta flower petals (ELTFP) was determined by the acute paw oedma 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 esterfied lutein from Tagetes erecta flower petals (ELTFP) was weighed and it was made into the suspension by using the Hydroxyl Propyl Methyl Cellulose(HPMCE5) 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-inflammed paw for comparison.
One rat is was kept as the 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 Diabetic Activity

The present investigation indicate that esterfied lutein from *Tagetes erecta* flower petals (ELTFP) shows siginificant 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 rats 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.

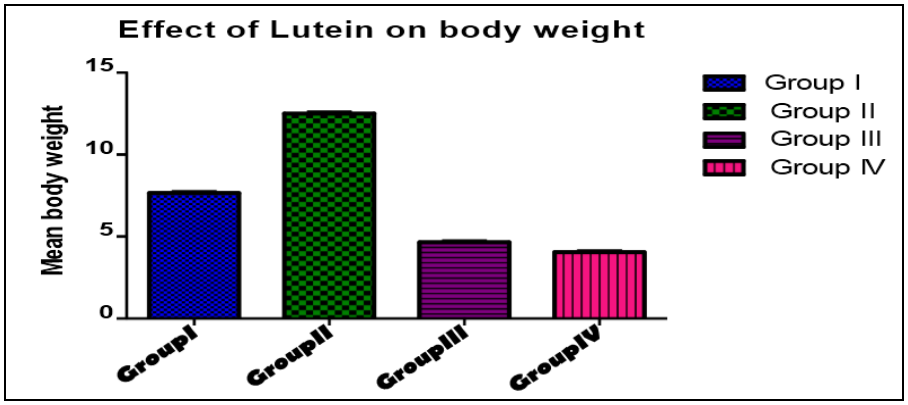
Result on body weight

Table 3: 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 *Tagete erecta* flower petals (ELTFP) 80mg/kg body weight orally



The mean deviation <0.05

Fig 3: Effect of Esrerified Lutein on body weight of diabetic rats

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

Result on Fasting blood glucose levels

Table 4: 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

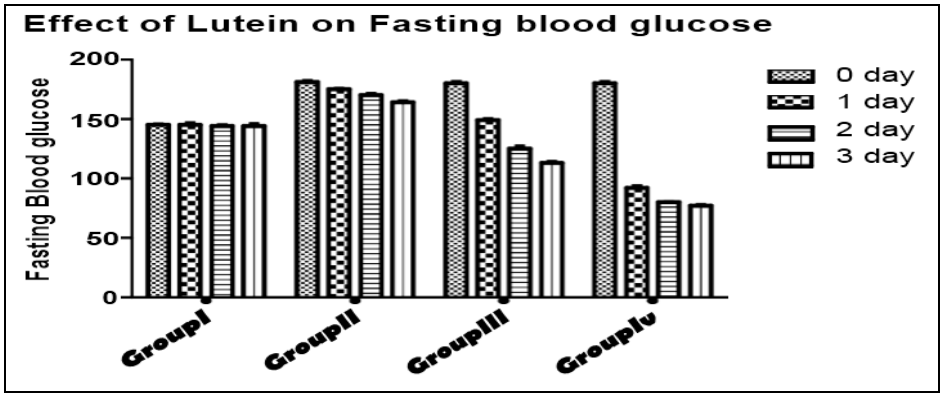


Fig 4: Effect of Esterified Lutein on fasting blood glucose levels of diabetic rats

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 erecta* flower petals**(ELTFP)** 80mg/kg body weight orally

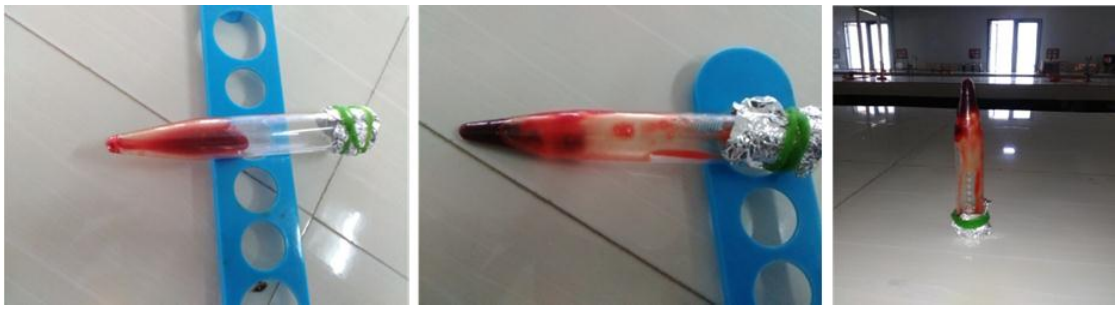
Wound Healing Activity

Control **Test** **Standard**

Fig 4: Effect of Esterified lutein on Angiogenesis

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

In-vitro Coagulant Activity



EDTA + Blood EDTA+Blood+ELTFP Blood

Fig 5: Effect of Esterified lutein on blood coagulant activity

Table 5: 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

*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)

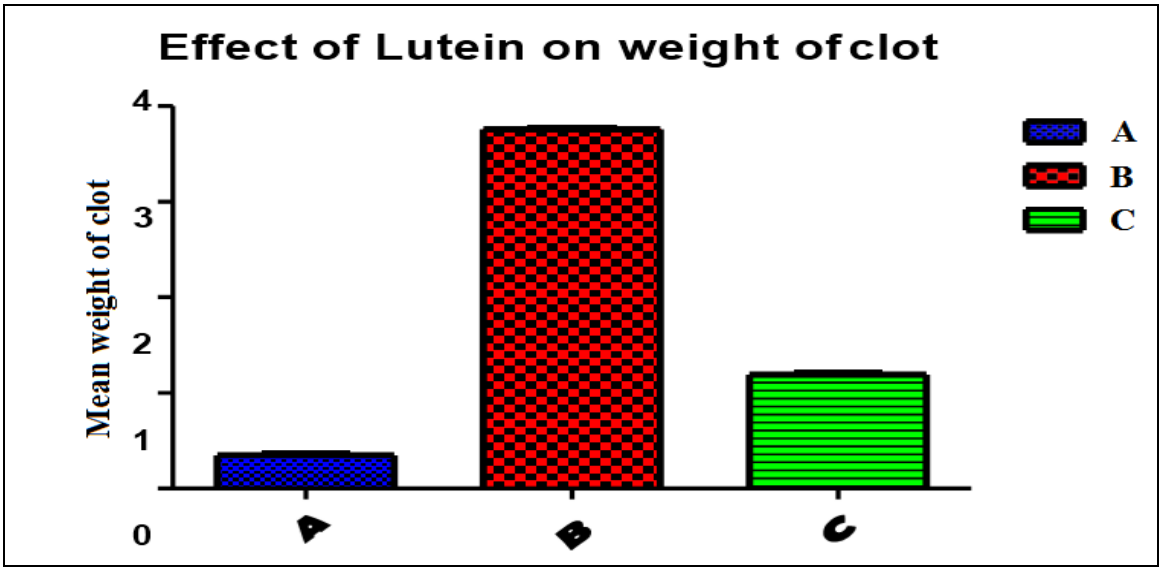


Fig 5: Effect of Esterified Lutein on weight of clot

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).

Anti-inflammatory Activity

Table 6: 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 esterfied lutein from *Tagetes erecta* flower petals (ELTFP) suspension.

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

Effect Lutein on Inflammation

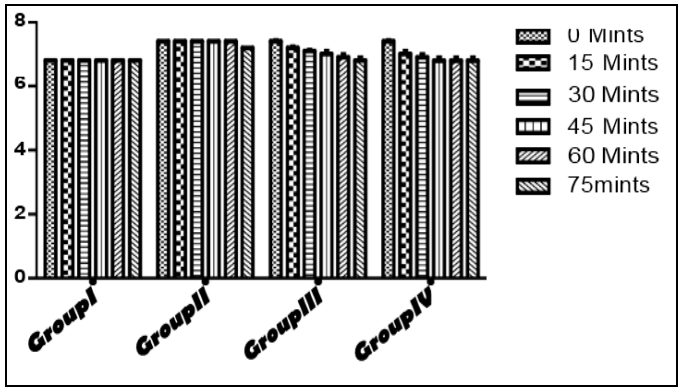


Fig 6: Effect of Esterified Lutein on Inflammation

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 esterfied lutein from *Tagetes erecta* flower petals (ELTFP) suspension.

Conclusion

Research is an 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.

Acknowledgement

It is our great pleasant duty to express our deep sense of gratitude and indebtedness to our beloved principal, Dr. S. Vidyadhara, Professor & Principal, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, to our guide Dr. R.L.C. Sasidhar, and to our co-guide D. Suryanarayana Raju, who have not only suggested this problem and guided all through the work but always keep my spirit high with his valuable suggestions and constant encouragement.

We are highly grateful to Dr. K. Basavapunnaiah, President, Dr. C.N. Srinivas, Secretary & Correspondent, Sri Ch. Narendranath, Vice- President and Sri J. Murali Mohan, Vice-President of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur, and to all life members of Nagarjuna Educational Society [NES], Guntur for providing the necessary facilities and co-operation.

We are greatly indebted to Dr. J. Ramesh Babu, Dr. R. Hari Babu, Sri. J. Venkateswara Rao, Dr. A. Ramu, Sri. S. Siva Prasad, Sri. J. Subba Rao, Dr. T.N.V. Ganesh Kumar, Smt. P. Viijetha, Sri. Ch. Aruna Kumar, Sri. B. Venkateswara Rao, Sri. B. Praveen Kumar, Sri. T. Bala Krishna, Smt. P. Vineeela, Sri. K. Vishwanath, Sri. N. Venkata Deepak, Sri. T. Deleep Kumar, Sri. D. Sandeep, Sri. S. Vikas, Sri. P. Satyaprasanna, Sri. P. Srinivasulu, Sri. B. Nagaraju, Smt. B. Sulochana, Dr. M. Raghava Kalayan, Smt. Sowjanya Lakshmi, Ms. J. Suhasini, Ms.D. Lakshmi Harika, and all the non-teaching staff of our esteemed college, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Guntur for giving their valuable suggestions for successful completion of project work.

We are thankful to V. Sindhura, E. Abhishek Chowdary of our class for their assistance in the work.

We would like to conclude our acknowledgements by recording my deep sense of affection to our parents, for their unabated encouragement and cheerful company throughout this endeavor, without which this work would have not been completed in stipulated period.

Place: Guntur

Date: May J. Sai Sugun (Y12PH0931)
K. Adilakshmi (Y12PH0934)
K. Snehapriya (Y12PH0937)
K. Gowthami Aishwarya (Y12PH0939)

References

1. Ambrogi A, Eggers R. Extraction and concentration of xanthophylls pigments from marigold (*Tagetes erecta*). In: Fourth Italian Conference on Supercritical Fluids and their applications, Italy, 1997, 129-133.
2. Bone RA, Landrum JT, Taris SL. Preliminary identification of the human macular pigment. Vision Res. 1985; 25:1531-1533.
3. Bown D. Encylopedia of herbs and their uses: Dorling Kinderslet : London, UK. 1995, 358-359.
4. Chew BP, Wong MW, Wong TS. Effects of lutein from marigold extract on immunity and growth of mammary tumours in mice. Anticancer Res. 1996; 16:3689-3694.
5. Crank J. The mathematics of diffusion. Oxford:oxford University Press, 1975.
6. Delgado-Varges F. Natural Colorants for Food and Nutraceutical Uses; CRC Press: Boca Raton FL. 2003, 42.
7. FAO. Guide to specifications.Food and Agriculture Organisation of the United Nations (FAO); Rome. FAO Food and Nutrition Paper, 1991, 5(2).
8. Fullmer LA, Shao A. The role of lutein in eye health amd nutrition. Am. Assoc. Cereal Chem. 2001; 46(9):408-413.
9. Gekas V. Mass transfer modeling. Journal of food enginerring. 2001; 49:97-102.
10. Handelman GJ. The evolving role of carotenoids in human biochemistry, Nutrition. 2001; 17:818-822.
11. Hendry GAF. Natural food colorants, (2nd ed.). London, UK: Chapman & Hall, 1996, 200-210.
12. Hencken H. Chemical and physiological behavior of feed carotenoids and their effects on pigmentation. Poult. Sci. 1992; 71:711-717.
13. http://www.who.int/diabetes/en/

14. Jose Luis Navarrete-Bolanos, *et al.* Improving Xanthophyll Exracrtion from Marigold Flower Using Cellulolytic Enzymes.
15. Khachik F, Steck A, Pfander H. Isolation and structural elucidation of (13Z, 13'Z, 3'R, 6'R)-Lutein from Marigold flowers, Kale,and human plasma.J Agric Food Chem. 1999; 47:455-481.
16. Krieienbuhl P, Rudin P, Rudolph W. Method of making carotenoids. U.S Patent. 2000; 6:150-561.
17. Krinsky NI, Russett MD, Handelman GJ, Snodderly DM. Structural and geometrical isomers of carotenoids in human plasma. J Nutr. 1990; 120:1654-1662.
18. Li W, Gao Y, Zhao J, Wang Q. Phenolic, Flavonoid and lutein ester content and anti-oxidant activity of 11 cultivars of Chinese marigold. J Agric. Food chem. 2007; 55:8478-8484.
19. Lu Y, Foo LY. Anti oxidant and radical scavenging activities of polyphenols and antioxidant activity in functional food. J Food Eng. 2006; 74:416-423.
20. Madhavi DL, Kagan DI. Process for the isolation of mixed carotenoids from plants. United States Patent. 2002; 6:380-442.
21. Masa Hojnik *et al.* Extraction of lutein from Marigold flower petals - Experimental kinetics and modeling, 2002.
22. Noor A Lokman, *et al.* Chick Chorioallantoic membrane (CAM) Assay as an in vivo model to study the effect of newly identified molecules on ovarian cancer invasion and metastasis.
23. Rice-Evans CA, Sampson J, Bramley PM, Holloway DE. Why do we expect carotenoids to be antioxidants *in vivo*? Free Rad Res. 1997; 26:381-398.
24. Richard Cantrill, Lutein from Tagetes erecta.
25. Subagio A, Naofumi M. Prooxidant activity of lutein and its dimyristate esters in corn triacylglyceride, Food Chem. 2003; 81:97-102.
26. Sugata Bhattacharya *et al.* Lutein Content and Invitro Antioxident activity of Different cultivars of Indian Marigold Flower (*Tagets patula* L.) Extract.
27. Tyczkowski JK, Hamilton PB. Altered metabolism of carotenoids during aflatoxicosis in young chickens. Poult. Sci. 1987; 66:1184-1188.
28. Vasudevan P, Kashyap S, Sharma S. Tagetes a Multipurpose plant, Bioresour,Technol.1997; 62:29-35.
29. Reha VBP. A Review on Piper betlel: Nature's promising medicinal reservoir.
30. Wei Li *et al.* Phenolic, Flavanoid, and Lutein Ester Content and Antioxident Activity of 11 Cultivars of Chinese Marigold.
31. Winter CA, Risley EA, Nus GN. Proc. soc. Exp. Biol. III:544-547,19.