



Phytochemical investigation and evaluation of anti-inflammatory and analgesic activity of *Tagetes erecta*

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Abstract

The aim of the present research work is to evaluate pharmacognostic features, anti-inflammatory and analgesic activity of *Tagetes erecta*. In the present study, Micro and Macroscopic features of fresh and dried root samples were explored and colour, shape, size, odor, and surface features had been observed from the root and then powder root material of *Tagetes erecta* plant. Light electron microscope i.e. Olympus CX-21i trinocular Microscope images of cross section of root and powdered root revealed that the existence of cork cells, Xylem fibers with tapered ends, lignified xylem vessels, phloem fibers, medullary rays, sclerides and parenchymatous cells. Phytochemical testing revealed the existence of flavonoids, alkaloids, tannins, phenols, steroids, acid compounds, glycosides, amino acids, and proteins. Methods: Rats Were Divided into 10 Groups of 6 Animals of Each. The Anti-inflammatory Activity Was Studied with Carrageenan Induced Rat Paw Edema Models. The analgesic activity was evaluated using tail flick and eddy's hot plate model. The ethanolic extract of *Tagetes erecta* whole plant preparations were compared with indomethacin. Physicochemical parameters including moisture content, ash value, extractive value and fluorescent behavior of root powder had been identified and ethanolic extract of *Tagetes erecta* suppresses the first phase of carrageenan-induced paw edema.

Keywords: tandardization; phytochemicals, *Tagetes erecta*

Introduction

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Natural products and their derivatives represent more than 50 % of all the drugs in clinical use in the world. The principles of Ayurvedic medicine and the medicinal uses of plants are contained in thousands of poetic hymns in the Rig Veda. It is a fact that the village people and tribes are generally relying on the medicine developed from the plants directly or indirectly. The WHO has estimated eighty per cent of the global population relying chiefly on traditional medicine [1]. In the beginning of 21st century phyto medicines achieved the reliability to rescue the patients from the fatal diseases. Herbal medicines have many advantages: have no side effects, better patient's tolerance and relatively less expensive. The chemical compounds of allopathic medicines are costly and have side effects. The patients are seeking the alternative system of medicines which are less expensive and will have no side effects. Research on medicinal plants is an important fact of biochemical research [2] in India because of several reasons. Inflammation is a disorder involving localized increases in the number of leukocytes and a variety of complex mediator molecules. Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs [3]. Because existing synthetic molecule like non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors that increase the

incidence of adverse cardiovascular thrombotic effects [4]. So, in order to overcome, there is need to focus on the scientific exploration of herbal drugs having fewer side effects. Despite the progress made in medical research during the past decades, the treatment of many serious diseases is still problematic. Chronic inflammatory diseases remain one of the world's major health problems [5, 7]. Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release and extravasations of fluid, cell migration, tissue breakdown and repair [8, 9]. Inflammation has become the focus of global scientific research because of its implication in virtually all human and animal diseases. The conventional drugs used to ameliorate this phenomenon are either too expensive or toxic and not commonly available to the rural folks that constitute the major populace of the world [10, 12]. Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli [13]. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases [14]. Currently used anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary.

Material & Methods

Selection and collection of plant material

Plants i.e., *Tagetes erecta* Whole part were searched in Madhya Pradesh particularly areas near to Indore. Plant was collected from area near Indore. Plants were collected as whole and herbarium was also prepared and submitted.

Plant was authenticated by Dr.Zia Ul Hasan, Botanist, Department of Botany, Safia College of Science, Bhopal MP [Voucher No. *Tagetes erecta*] were submitted to department. Plant materials was washed and dried under shade. Dried plant material was grinded using electric grinder at Department of Pharmacy, Dr. A.P.J. Abdul Kalam University, Indore, MP. Dried plant material was kept in closed air tight container till any further use.
Tagetes erecta 321/BOT/SAFIA/18

Morphological Studies

Organoleptic evaluation

Colour
 Odour
 Texture

Microscopic examination

Epidermal layer
 Trichomes
 Vascular bundle
 Stomata

Physiochemical examination

Foreign matter

The sample shall be free from visible signs of mold growth, sliminess, stones, rodent excreta, insects or any other noxious foreign matter when examined as given below. Took a representative portion from a large container, or remove the entire contents of the packing if 100 g or less, and spread in a thin layer in a suitable dish or tray. Examine in daylight with unaided eye. Transferred suspected particles, if any, to a petri dish, and examine with 10x lens in daylight.

Ash value

Total ash

Place about 2-4g of the ground air-dried material, accurately weighed, in a previously ignited and tared crucible (usually of platinum or silica). Spread the material in an even layer and ignite it by gradually increasing the heat to 500-600°C until it is white, indicating the absence of carbon. Cool in a desiccator and weigh.

If carbon-free ash cannot be obtained in this manner, cool the crucible and moisten the residue with about 2 ml of water or a saturated solution of ammonium nitrate R. Dry on a water-bath, then on a hot-plate and ignite to constant weight.

Allow the residue to cool in a suitable desiccator for 30 minutes, then weigh without delay. Calculate the content of total ash in mg per g of air-dried material.

Acid insoluble ash

To the crucible containing the total ash, add 25 ml of hydrochloric acid (~70g/l) TS, cover with a watch-glass and boil gently for 5 minutes. Rinse the watch-glass with 5 ml of hot water and add this liquid to the crucible. Collect the insoluble matter on an ashless filter-paper and wash with hot water until the filtrate is neutral. Transferred the filter-paper containing the insoluble matter to the original crucible, dry on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes, then weigh without delay. Calculate the content of acid-insoluble ash in mg per g of air-dried material.

Water soluble ash

To the crucible containing the total ash, add 25 ml of water and boil for 5 minutes. Collect the insoluble matter in a sintered-glass crucible or on an ashless filter-paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per g of air-dried material

Sulphated ash

Heat a silica or platinum crucible to redness for 10 minutes, allow to cool in a desiccator and weigh. Put 1 to 2 g of the substance, accurately weighed, into the crucible, ignite gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1 ml of *sulphuric acid*, heat gently until white fumes are no longer evolved and ignite at 8000 ± 250 until all black particles have disappeared. Conduct the ignition in a place protected from air currents. Allow the crucible to cool, add a few drops of *sulphuric acid* and heat. Ignite as before, allow to cool and weigh. Repeat the operation until two successive weighing do not differ by more than 0.5 mg.

Loss on drying

Place about 10 g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tared evaporating dish. For example, for unground or unpowdered drug, Prepared about 10 g of the sample by cutting shredding so that the parts are about 3 mm in thickness. Seeds and fruits, smaller than 3 mm should be cracked. Avoid the use of high speed mills in preparing the samples, and exercise care that no appreciable amount of moisture is lost during preparation and that the portion taken is representative of the official sample. After placing the above said amount of the drug in the tared evaporating dish, dry at 105° for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference.

Extractive value

Ethanol soluble extractive value

Macerate 5 g of the air-dried drug, coarsely powdered, with 100 ml of alcohol the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug. For determination of methanol soluble extractive use methanol on place of alcohol.

Water soluble extractive value

Proceed as directed for the determination of alcohol-soluble extractive, using *chloroform-water* instead of ethanol.

Crude extract preparation

In present study, plant materials were extracted by continuous hot percolation method using Soxhlet apparatus.

Powdered material of *Tagetes erecta* was placed in thimble of Soxhlet apparatus. Soxhlation was performed at 60°C using petroleum ether as non-polar solvent. Exhausted plant material (marc) was dried and afterward re-extracted with ethyl acetate and methanol solvent. For each solvent, soxhlation was continued till no visual colour change was observed in siphon tube and completion of extraction was confirmed by absence of any residual solvent, when evaporated. Obtained extracts was evaporated using rotary vacuum evaporator (Buchi type) at 40°C. Dried extract was weighed and percentage yield for each extract was determined using formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Prepared extracts were observed for organoleptic characters (percentage yield, colour and odour) and were packed in air tight container and labelled till further use (The Ayurvedic Pharmacopoeia of India).

Screening of phytochemical constituents

Qualitative phytochemical testing of extracts was done to study the presence or absence of various phytochemical constituents using standard tests (Kokate CK *et al.*, 1993).

Tests for Carbohydrates

Molish Test: 2 ml of aqueous extract was treated with 2 drops of alcoholic α -naphthol solution in a test tube and then 1 ml of conc. H_2SO_4 was added carefully along the sides of the test tube. Formation of violet ring at the junction indicated the presence of carbohydrates.

Fehling's Test: To 1 ml of aqueous extract, 1 ml of Fehling's A and 1 ml of Fehling's B solutions were added in a test tube and heated in the water bath for 10 minutes. Formation of red precipitate indicated the presence of reducing sugar.

Benedict's test: Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.

Tests for Protein and Amino acids

Biuret's Test: The extract was treated with 1 ml of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulphate solution was added to the above mixture. The formation of violet or pink color indicated the presence of proteins.

Million's Test: 3 ml of extract was mixed with 5 ml of Million's reagent. White precipitate formed which on heating turned to brick red, indicated the presence of proteins.

Ninhydrin Test: 3 ml of the test solution was heated with 3 drops of 5% Ninhydrin solution in a water bath for 10 minutes. Formation of blue color indicated the presence of amino acids.

Tests for Glycosides

Borntrager's Test: To 3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added mixed well. The organic solvent layer was separated and ammonia was added to it. Formation of pink

to red color in ammonical layer indicated presence of anthraquinone glycosides.

Legal's Test: 1 ml of test solution was dissolved in pyridine. 1 ml of sodium nitropruside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red color indicated the presence of cardiac glycosides.

Keller-Killiani Test: To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Formation of blue color in the acetic acid layer indicated the presence of cardiac glycosides.

Tests for Alkaloids

To the extract, dilute hydrochloric acid was added; further the solution was mixed well and filtered. With the filtrate, the following tests were performed:

Mayer's Test: To 2-3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of white or creamy precipitate indicated the presence of alkaloids.

Dragendroff's Test: To 1-2 ml of filtrate, few drops of Dragendroff's reagent were added in a test tube. Formation of red precipitate indicated the presence of alkaloids.

Hager's Test: To 1-2 ml of filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow color precipitate indicated the presence of alkaloids.

Wagner's Test: To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish brown precipitate indicated the presence of alkaloids.

Tests for Saponins

Froth Test: The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicated the presence of saponins.

Tests for Flavonoids

Lead Acetate Test: The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate may indicate the presence of flavonoids.

Alkaline Reagent Test: The extract was treated with few drops of sodium hydroxide separately in a test tube. Formation of intense yellow color, which becomes color less on addition of few drops of dilute acid, indicated presence of flavonoids.

Shinoda test: To the extract, 5 ml (95%) of ethanol was added. The mixture was treated with few fragments of magnesium turning, followed by drop wise addition of concentrated hydrochloric acid. Formation of pink color indicated presence of flavonoids.

Tests for Triterpenoids and Steroids

Salkowski's Test: The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layers turns red, sterol are present. Presence of golden yellow layer at bottom indicated the presence of triterpenes. **Liebermann-Burchard's Test:** The extract was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled. Concentrated sulphuric acid was added through the side of the test tube. Formation of brown ring at the junction of two layers, if upper layer turned green, indicated the presence of steroids

and formation of deep red color indicated the presence of triterpenoids.

Tests for Tannin and Phenolic Compounds

Ferric Chloride Test: Some amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicated the presence of phenolic compounds.

Lead Acetate Test: Some amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. Formation of white precipitate indicated the presence of phenolic compounds.

Dilute Iodine Solution test: To 2-3 ml of extract, few drops of dilute iodine solution were added. Formation of transient red color indicated the presence of phenolic compounds.

Gelatin Test: Some quantity of extract dissolved in distilled water. To this solution 2 ml of 1% gelatin solution containing 10% sodium chloride was added. Development of white precipitate indicated the presence of phenolic compounds.

Tests for Fats and Oils

Solubility test: To 2-3 ml of the alcoholic solution of extract, few ml of chloroform was added and solubility was observed, or to 2-3 ml of the alcoholic solution of extract, few ml of 90% ethanol was added and solubility was observed.

Acute oral toxicity (OECD 423)

The acute toxic class method set out in this Guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund

status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e., no further testing is needed, dosing of three additional animals, with the same dose and, dosing of three additional animals at the next higher or the next lower dose level. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight.

In vivo Carrageenan-Induced Paw Edema

Edema in the left hind paw of rat was induced by injection 0.05 ml of 1% (w:v) carrageenan (Sigma, St Louis, MO) in saline into the footpad, subcutaneously. The paw diameter were determined using a digital vernier calliper prior to and 1, 3 and 5 h after carrageenan injection. The drug test groups were treated with Extract (200 and 400 mg/kg body weight, P.O.) 1 h before carrageenan injection. The animals in the control group received saline only. Another group of rats was administered with indomethacin (10 mg/kg, ip.) in Distilled water as a standard reference.

The anti-inflammatory activity was calculated as percentage inhibition of Carrageenan induced paw edema using the following formula.

$$\text{Percent inhibition} = [1 - \frac{\text{paw diameter in treated (dt)}}{\text{paw diameter in control (dc)}}] \times 100.$$

Table 1: Treatment group and doses for Carrageenan-Induced Paw Edema

S. no.	Groups	Treatment
1	Control group	1% Carrageenan solution (5 ml /kg b.w)
2	Standard group	Carrageenan + Indomethacin (10 mg/kg b.w)
3	Treatment group-III	Carrageenan + TE Extract (200 mg/kg), orally
4	Treatment group-IV	Carrageenan + TE Extract (400 mg/kg), orally

Screening Method for Analgesic Activity

Tail flick method

Weigh and divide the mice in five groups. Group I will work as vehicle treated, group II will work as standard drug treated, Group III, IV will receive extracts of selected plants. The reaction time will get record by using tail flick Analgesiometer at different time interval after the drug administration.

Table 2: Treatment group and doses for Tail flick method & Eddy's Hot Plate Method

S. no.	Groups	Treatment
1	Control	10 ml/kg b.w
2	Standard	Diclofenac sodium (10 mg/kg b.w)
3	Group-III	TE Extract (200 mg/kg), orally
4	Group-IV	TE Extract (400 mg/kg), orally

Eddy's Hot Plate Method

Weigh and divide the mice in five groups. Group I will work as vehicle treated, group II will work as standard drug treated, Group III, IV will receive extracts of selected plants. The latency to flick the hind paw or lick or jump from the hot plate is the reaction time here. The reaction time will get record by using Eddy's hot plate instrument at different time interval after the drug administration.

Result and Discussion

Morphological studies



Fig 1: Morphological features of *Tagetes erecta* Flowers



Fig 2: Morphological features of *Tagetes erecta* Flowers

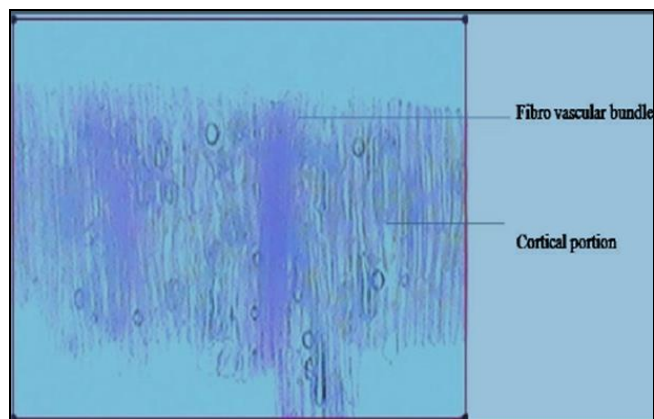


Fig 3: Transverse section (calyx) *Tagetes erecta* Flowers

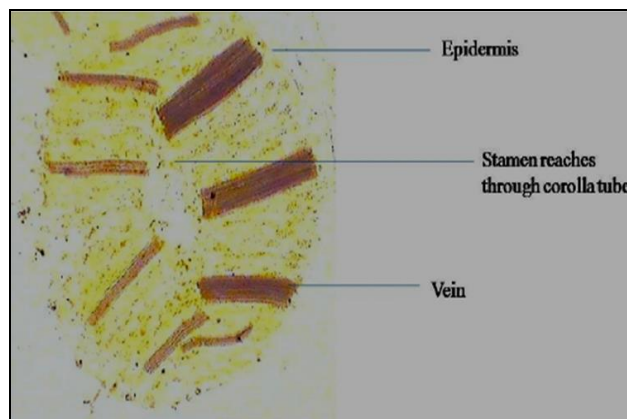


Fig 4: Transverse section (corolla tube) *Tagetes erecta* Flowers

Physicochemical examination

Table 3: Physicochemical evaluation

Parameters.	% w/w
FOM	0.52±0.18
Total Ash	4.21
Acid Insoluble	0.56
Water soluble	2.64
Sulphated ash	3.54
LOD	7.32±0.12
SI	2.14±0.12
Crude fibre yield	12.35±0.37
Ethanol EV	13.72±1.22
Water EV	12.41±1.46
% Yield	11.54

Screening of phytochemical constituents in *Tagetes erecta* extracts

Table 4: Phytochemical constituents in *Tagetes erecta* extracts

S. no.	Test methanol	Result			
		PETE	EATE	CHTE	METE
<i>Test for carbohydrates</i>					
1	Molish test	Negative	Negative	Negative	Negative
<i>Test for reducing sugars</i>					
2	Fehling's test	Negative	Negative	Negative	Negative
3	Benedict's test	Negative	Negative	Negative	Negative
<i>Test for monosaccharides</i>					
4	Barfoed's test	Negative	Negative	Negative	Negative
<i>Test for proteins</i>					
6	Biuret test	Positive	Positive	Negative	Negative
7	Millon's test	Negative	Negative	Negative	Negative
8	Xanthoprotein test	Negative	Negative	Negative	Negative
<i>Test for steroids</i>					
9	Salkowski's test	Negative	Negative	Negative	Negative
10	Liebermann-burchard reaction	Negative	Negative	Negative	Negative
<i>Test for glycosides:</i>					
<i>Test for cardiac glycosides</i>					
11	Baljet's test	Positive	Positive	Positive	Positive
12	Legal's test	Negative	Positive	Positive	Positive
13	Keller-killiani test	Negative	Negative	Positive	Positive
<i>Test for anthroquinone glycosides</i>					
14	Borntrager's test	Negative	Positive	Positive	Negative
<i>Test for saponin glycosides</i>					
15	Foam test	Positive	Positive	Positive	Negative
16	Heamolytic test	Positive	Positive	Positive	Negative
<i>Test for flavonoids</i>					
17	Shinoda's test	Positive	Positive	Positive	Negative
<i>Test for fats and oils:</i>					
Solubility test					

18	Chloroform	Positive	Positive	Positive	Positive
19	90% ethanol	Positive	Positive	Positive	Positive
Test for tannins and phenolic compounds					
20	Ferric chloride test:	Positive	Positive	Positive	Positive
21	Lead acetate test:	Negative	Negative	Negative	Negative
Test for alkaloids					
22	Dragendorff's test	Positive	Positive	Positive	Positive
23	Mayer's test	Positive	Positive	Positive	Positive
24	Hager's test	Positive	Positive	Positive	Positive
25	Wagner's test	Positive	Positive	Positive	Positive
Test for amino acids					
26	Ninhydrin test	Negative	Negative	Positive	Negative

In-vivo Experiment

Table 5: Showing results of acute oral toxicity (OECD)

Plant used	Group no.	Dose (mg/kg body weight)	Observations/mortality
<i>Tagetes erecta</i>	TE-I	5 mg/kg	0/3
	TE-II	50 mg/kg	1/3
	TE-III	300 mg/kg	0/3
	TE-IV	2000 mg/kg	0/3

In case of acute oral toxicity study when animals were treated with 5, 50, 300, and 2000 mg/kg b.w. of dose.

There was no mortality and any behavioural changes thus selected dose will be on the basis of LD50, 200 and 400 mg/kgb.w.

Carrageenan-Induced Paw Edema

The anti-inflammatory activity of *Thunbergia grandiflora* against carrageenan induced paw edema has been shown in and the results were comparable to that of standard drug indomethacine, a pro-type of non-steroidal anti-inflammatory agent. The *Thunbergia grandiflora* extract showed maximum inhibition of 63.79 at the dose 400 mg/kg body wt. after extract treatment in carrageenan induced paw edema whereas the reference drug (indomethacine) produced 82.17% of inhibition.

Table 6: Results carrageenan-induced paw edema

Treatment groups	Paw volume		
	1 hr	3 hr	5 hr
Control	1.567±0.304	1.780±0.278	2.395± 0.199
Standard (Indomethacine)	0.770±0.111	0.667±0.104	0.427± 0.073
Treatment group-VII	1.367±0.304	1.850±0.278	2.105± 0.199
Treatment group-VIII	0.890±0.111	0.757±0.104	0.537± 0.073

Table 7: Results of analgesic activity

Groups	Dose	Reaction time in sec (Results of Tail flick method)					
		0 min	30 Min	60 Min	90 min	120 Min	180 min
Control	10 ml/kg	2.00±0.258	3.00±0.258	3.60±0.211	4.50±0.341	5.50±0.341	6.30±0.333
Standard Diclofenac sod.	10 mg/kg	3.16±0.166	4.16±0.307	4.50±0.342	5.16±0.401	6.33±0.557	7.00±0.258
Group-VII	TE 200 mg/kg	2.85±0.152	3.24±0.441	3.76±0.222	4.56±0.855	4.85±0.527	5.21±0.153
Group-VIII	TE 400 mg/kg	3.21±0.241	3.84±0.248	4.21±0.425	4.45±0.417	4.89±0.124	5.45±0.195
Groups	Dose	Reaction time in sec (Results Eddy's Hot Plate Method)					
		0 min	30 Min	60 Min	90 min	120 Min	180 min
Control	10 ml/kg	4.00±0.258	4.83±0.166	5.33±0.211	6.33±0.211	6.50±0.224	7.33±0.211
Standard Diclofenac Sodium	10 mg/kg	5.16±0.166	5.50±0.224	6.33±0.211	7.16±0.211	7.50±0.224	8.16±0.307
Group-VII	TE 200 mg/kg	4.85±0.152	5.24±0.441	5.76±0.222	6.56±0.855	6.85±0.527	7.21±0.153
Group-VIII	TE 400 mg/kg	5.21±0.241	5.84±0.248	6.21±0.425	6.45±0.417	6.89±0.124	7.45±0.195

Conclusion

Phytochemical studies on *Tagetes erecta* plants indicate it to be a useful plant to investigate for phytochemical and biological assays. HPLC analysis may serve as a useful data for the standardization of the drug. The data generated from this study would help in the authentication of various parts of *Tagetes erecta* a very important constituent of various herbal drug formulations.

This may lead to easier authentication of herbal drugs procured from markets for the correct identification of the medicinal plant ingredients. The Present Results Suggest That Ethanolic Extract of *Tagetes erecta* Suppresses The First Phase Of Carrageenan-Induced Paw Edema, Thus, Confirming an Nsaid-Like Property.

References

- Rang Hp, Dale Mn, Ritter Jm, Henderson G. Analgesic Drugs. In: Rang And Dale's Pharmacology. 7th Ed. Edinburgh: Elsevier Churchill Livingstone, 2012:503-524.
- Cole Be. Pain Management: Classifying, Understanding and Treating Pain. Hospital Physician, 2002, 23-30.
- Ferrero-Miliani L, Nielsen Oh, Anderson Ps, Girardin Se. Chronic Inflammation: Importance of Nod2 and Nalp3 in Interleukin-1beta Generation. Clin. Exp. Immunol, 2007;147(2):227-35.
- Anderson Wad. Inflammation and Healing. Pathology, 9th Edition, C.V. Mosby Co, 1990, 1(67).
- Walker Sm. Introduction of Pain. In: Macintyre Pe, Walker SM, Rawbotham Dj, Editors. Clinical Pain Management: Acute Pain. 2nd Ed. Hodder & Stoughton: London, 2008, 20-31.
- Rang Hp, Dale Mm, Ritter Jm, Henderson G. Anti-Inflammatory and immune suppressant Drugs. In: Rang And Dale's Pharmacology, 7th Edition. Edinburgh: Elsevier Churchill Livingstone, 2008, 318-34.
- Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey Of Chronic Pain In Europe; Prevalence, Impact On Daily Life and treatment. Eur J Pain, 2006;10:287-333.

8. Wane Jr, Bakhle Ys, Botting Rn. Cyclooxygenases 1 and 2. Annual Review Of Pharmacology and Toxicology,2015:38:97-120.
9. Cks, Lirk P, Tan Ch, Seymour Ra. Evidence-Based Update On Nonsteroidal Anti-Inflammatory Drugs. Clin Med Res,2007:5(1):19-34.
10. World Health Organization. Who's Pain Ladder. Geneva, Switz: World Health Organization, 2010.
11. Watanabe S, Bruera E. Corticosteroids as adjuvant analgesics. J Pain Symptom Manage,1991:9(7):442-5.
12. Sengupta R, Sheorey Sd, Hinge Ma. Analgesic and anti-Inflammatory Plants: An Updated Review. International Journal of Pharmaceutical Sciences Review and Research,2012:12(2):114-19.
13. Kasture Sb, Kasture Vs, Chopde Ct. Anti-Inflammatory Activity of Rubia Cordifolia Roots. Journal of Natural Remedies,2001:1(2):111-15.
14. Joharapurkar Aa, Deode Nm, ZambadSp, Umathe Sn. Immunomodulatory Activity of Alcoholic Extract of Rubia Cordifolia Linn. Indian Drugs,2003:40(3):179-81.
15. Neelam C, Ranjan B, Komal S, Nootan C. Review On Cassia Fistula. Int J Res AyurPharmacy,2011:2(2):426-30.
16. Mohan H. In: Harsh Mohan's Text Book of Pathology. 6th Edition. New Delhi: Jaypee Brothers Medical Publishers Private Limited, 2010.
17. Suresha Rn, Amoghmath S, Vaibhavi Ps, Shruthi Sl, Jayanthi Mk, Kalabharathi Hl. Evaluation Of Analgesic Activity Of Perindopril In Albino Mice. J Adv Pharm Technol Res,2014:5(3):129-33.
18. Radwan Maa, RagabEa, SabryNm, El-Shenawy Sm. Synthesis and Biological Evaluation of new 3-Substituted Indole Derivatives as Potential Anti-Inflammatory and Analgesic Agents. Bioorg. Med Chem,2007:15:3832-41.
19. Parmar N, Rawat M, Kumar T. Evaluation of Anti-Inflammatory Potential of Kigelia Pinnata Leaf Extract in Wistar Rats. Asia NJ Pharm Clin Res,2012:5(1):95-7.
20. Winter Ca, Porter Cc. Effect of Alterations In The Side Chain Upon Anti-Inflammatory and Liver Glycogen Activities Of Hydrocortisone Esters. J Am Pharm Assoc Sci Educ,1957:46:515-19.
21. Spector Wg. The Granulomatous Inflammatory Exudate. Int Rev Exp Pathol,1969:8:51-5.