International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC AND AQUEOUS LEAF EXTRACT OF *TROPAEOLUM MAJUS* L

Syed Mujtaba Ahmed[1], Anil Middha[1], Mohammed Omer[1], D. Ramakrishna[2]

[1]Deaprtment of Pharmacy, OPJS University, Churu, Rajasthan
[2]Sushrut Institute of Pharmacy, Taddanapalli, Pulkal, Medak, Telangana, India

***Corresponding author e-mail: mujju203@gmail.com**

ABSTRACT

In the present study ethanolic and aqueous leaf extract of *Tropaeolum majus* L was investigated for analgesic and anti-inflammatory activity. Analgesic activity was determined by three different methods (tail immersion, hot plate and writhing method), anti-inflammatory activity was determined by three different methods (carrageenan, histamine induced paw edema & cotton pellet granuloma) at dose 200,400mg/kg b.wt in experimental animals using diclofenac sodium, tramadol, Indomethacin as reference drugs. In all the animals models the results obtained were statistically significant ($p<0.05$) in comparison to control. The results obtained indicate that *Tropaeloum majus* L has significant analgesic and anti-inflammatory activities in those animal models.

Key words: *Tropaeolum majus*, analgesic activity, anti-inflammatory activity

INTRODUCTION

Pain is unpleasant sensation localized to a part of the body. It is both sensation and emotion. Pain usually occurs when peripheral nociceptors are stimulated in response to tissue injury, visceral distension or other factors. In such situation, pain perception is a normal physiologic response mediated by healthy nervous system [1].Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection it is incorrect to use the terms as synonyms, infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen [2].Most of the anti-inflammatory drugs are potent inhibitors of Cycloxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are hyperalgesic, potent vasodilators and also contribute to erythema, edema and pain. Hence for treating inflammatory diseases, analgesic and anti-inflammatory agents are required [3].Many plant products from natural sources are used for the treatment and prevention of diseases. Natural plant compounds are now gaining more pharmacological attention as many unexplored products are showing a wide range of pharmacological activities.

Tropaeolum majus L belongs to the family Tropaeolaceae. As medicinal plant it contains glucosinolates,a mustard oil glycoside called glycotropeoline which have antibiotic, antifungal, antiviral and antibacterial properties to treat infections, cold flu and digestive upsets[4]. *Tropaeolum majus* L are grown in summer and do well in full sunlight or light shade [5].This species of the family Tropaeolaceae contains a single glucosinolate benzylglucosinolate which is biosynthetically derived from L-Phe[6]. Phytochemical studies detected the presence of fatty acids, benzylisothiocynate and flavanoids in seeds and leaves of *Tropaeolum majus* L[7]. Tropaeolum majus have shown biological activities like diuretic and potassium sparing effect of isoquercitrin[8], antihypertensive effect[9], antimicrobial and anti-inflammatory activity of volatile oil[10]. The floral

biology of *Tropaeolum majus* L [11] has been reported. Therefore the present study was undertaken to investigate analgesic and anti-inflammatory activity of ethanolic and aqueous leaf extract of *Tropaeolum majus* L.

MATERIALS AND METHODS

Plant material: The leaf of *Tropaeolum majus* L was collected from chittoor district Andhra Pradesh in the month of June 2014.The plant was authenticated by Dr.K. Madhavachetty, Department of Botany, Sri Venkateswara University Tiruphati, and Voucher specimen of the leaf of the plant was kept in museum of OPJS.

Preparation of ethanolic leaf extract: The leaf of *Tropaeolum majus* L were shade dried for 3-5 days. Dried plant material was ground to coarse powder using a blender and stored at ambient temperature and passed through sieve no 20 and extracted in a soxhlet apparatus for two days using 90% alcohol. The extract was concentrated under reduced pressure using a rotary evaporator. The yield of the extract was found to be 9.8 %. Extract was preserved in a desicator until further use.

Preparation of aqueous leaf extract: The leaf of *Tropaeolum majus* L were dried in shade and powdered. The aqueous extract was prepared by cold maceration the powder was soaked in distilled water and stirred intermittently and then left for 7 days .Macerated leaf extract were filtered through coarse sieve and filtered. The filtrate was dried at reduced pressure in a rotary evaporator and freeze dried. The extract was used for further studies. The yield of the extract was found to be 13.4%.

Animals: Swiss albino mice of both sex weighing 25-30 g and wistar rats of both sex weighing 150-200 g were procured from National institute of Nutrition Hyderabad.. Animals were kept and maintained under laboratory condition at temperature 25 ± 2^{o}C, relative humidity 55 ± 10 % and 12:12 light: dark cycle. The animals were fed on standard pellet diet and ad libium and had free access to water. The experiments were performed after the approval of protocol by the institutional animal ethic committee (IAEC) and were carried out in accordance with current guidelines for the care of the laboratory animals.

Acute toxicity Studies: Acute toxicity studies were carried out for ethanolic & aqueous leaf extract of *Tropaeolum majus* L according to the reported procedure [12]

Analgesic Activity

Tail Immersion test: Albino rats of wistar strain weighing 150-200gms of either sex were divided into six groups of six animals used. The animals were kept in vertical position to hang the tail up to 5 cm , tail was introduced in hot water at temperature 55± 0.5^{0}C.The time in seconds to withdraw the tail out of water was taken as the reaction time. The cut-off time, i.e. time of no response was put at 30s.The reaction time was recorded with a stopwatch. The animals were treated with ethanolic & aqueous leaf extract of *Tropaeolum majus* L (200, 400 mg/kg b.wt), saline (vehicle) or standard drug (diclofenac sodium 50 mg/kg), were administered intraperitoneally 30 min before the immersion of the tail. The base line latency was measured before and after drug treatment in a regular interval of 0 min, 30 min, 60 min and 120 min [13].

Hot Plate method: Albino rats of wistar strain weighing 150-200gms of either sex were divided into six groups of six animals. *Tropaeolum majus* L ethanolic & aqueous leaf extract at dose (200mg/kg, 400mg/kg b.wt), saline (control) and tramadol (30mg/kg) was administered intraperitoneally. Animals in all groups were individually exposed to the hot plate method. Animals were acclimatized to laboratory conditions one hour before the start of the experiment with food and water available ad libitium. All drugs were given orally to the respective group rats as suspension in normal saline. Animals were subjected to pretesting on hot plate maintained at 55 ± 0.5^{0}C.Animals having latency time greater than 15 s on hot plate during pretesting (latency time) were rejected. The reaction time was taken in seconds for forepaw licking or jumping was taken. A cut off time + 10 s was followed avoiding thermal injury to the paws. The reaction time was recorded before and after drug treatment in regular interval of 0 min, 30 min, 60 min and 120 min following administration of test or standard drug [14].

Acetic Acid Induced writhing Test: Swiss albino mice of either sex weighing 25-30 g were divided into six groups of six animals. All animals were withdrawn from food 2h before the start of experiment. The mice were treated with *Tropaeolum majus* L ethanolic & aqueous leaf extract at dose (200mg/kg, 400mg/kg b.wt), saline (control) and diclofenac sodium (10mg/kg) was administered, 30 min before administration of acetic acid .Animals were treated i.p with 1% acetic acid. The number of abdominal constrictions (writhes) was counted after 5 min of acetic acid injection for the period of 10 min [15].

Anti-inflammatory activity:

Carrageenan induced paw edema: Albino rats of wistar strain weighing 150-200gms of either sex were used. *Tropaeolum majus* L ethanolic & alcoholic leaf extract at dose (200mg/kg, 400mg/kg b.wt), saline (control) and Indomethacin (10 mg/kg) were administered intraperitoneally. After 30 minutes to the above intraperitoneal administration, carrageenan (1% 0.05 ml) was injected subcutaneously in the sub plantar tissue of the right hind paw of each rat. The inflammation was measured using plethysmometer immediately after injection of carrageenan and then 1,2, 3,4 and 5h.The average foot swelling in drug treated animals as well as standard was compared with that of control [16].

Histamine Induced paw edema: Animals were divided as in the previous experiment and inflammation was induced by subcutaneous injection of 0.1 freshly prepared solutions of histamine (1mg/ml) into the hind paws of mice. The percent inhibition of paw edema induced by each test sample was calculated as described in case the carrageenan induced paw test [17].

Cotton pellet induced granuloma: Albino rats of wistar strain weighing 150-200gms of either sex were used. The animals received ethanolic & aqueous leaf extract of *Tropaeolum majus* L (200, 400mg/kg b.wt), Indomethacin (10mg/kg), saline (control) orally once a day through an oral cannula over seven consecutive days .Sub acute inflammation was produced by cotton pellet granuloma model in rats, on day 1, with aseptic precautions sterile cotton pellets (50± 1 mg) were implanted subcutaneously, along the flanks of axillae and groins bilaterally under ether anesthesia..The animals were sacrificed on the 8th day. The granulation tissue with cotton pellet was dried at 60^{0}C overnight and then the dry weight was taken. Weight of the cotton pellet before implantation was subtracted from weight of the dissected dried pellets. Only dry weight of the granuloma formed was used for statistical analysis [18].

ANALGESIC ACTIVITY:

Table 1: Analgesic activity of *Tropaeolum majus* L using tail immersion method in rats

Groups	Dose mg/kg b.wt	0 min	30 min	60 min	90 min	120 min
Control (Saline water)	10 ml	3.40 ± 0.244	3.43 ± 0.183	3.61 ± 0.088	3.22 ± 0.274	3.17 ± 0.316
Standard (Diclofenac sodium)	50 mg/kg	5.2 ± 0.093**	5.94 ± 0.257*	6.20 ± 0.202**	6.24±0.225**	6.26± 0.302**
Ethanolic Extract	200 mg	3.48 ± 0.231^{ns}	3.58 ± .162*	4.80 ± 0.395*	4.88 ± 0.397^{ns}	4.54 ± 0.335*
	400 mg	3.50 ± 0.196*	3.55 ± 0.212*	4.83± 0.232*	4.85 ± 0.226*	4.50 ± 0.327*
Aqueous Extract	200 mg	3.52 ± 0.199^{ns}	3.61 ± .215*	4.89 ± 0.226*	4.87 ± 0.225*	4.56 ± 0.229^{ns}
	400 mg	3.53 ± 0.182*	3.68± 0.262*	4.86± 0.213*	4.89 ± 0.224*	4.52 ± 0.293^{ns}

Values are mean ± SEM (n=6) when compared with control * P<0.05, **P<0.01, ***P<0.001 were considered significant comparing to control

Table 2: Analgesic activity of *Tropaeolum majus* L by hot plate method in rats

Groups	Dose mg/kg b.wt	0 min	30 min	60 min	90 min	120 min
Control(saline water)	10 ml	9.10 ± 0.280	9.18 ± 0.261	9.14 ±0.267	9.22 ± 0.201	9.08 ± 0.258
Standard (Tramadol)	30mg/kg	9.50 ± 0.431*	15.89± 0.371**	16.24± .281***	16.85± .142***	16.98 ± 0.186***
Ethanolic Extract	200 mg	9.20 ± 0.255*	11.55 ± 0.735*	13.78 ± 0.662*	13.14 ± 0.564*	13.10 ± 0.590*
	400 mg	9.33 ± 0.226*	12.74 ± 0.183*	14.10 ± 0.261**	13.98± 0.229*	13.90 ±0.226*
Aqueous Extract	200 mg	9.28 ± 0.220^{ns}	11.82 ± 0.282*	13.47 ± 0.416*	13.10 ± 0.411*	12.98± 0.410*
	400 mg	9.48 ± 0.261*	12.35± 0.183*	14.68 ± 0.249*	13.92 ± 0.506*	13.26 ±0.533*

Values are mean ± SEM (n=6) when compared with control * P<0.05, **P<0.01, ***P<0.001 were considered significant comparing to control

Table 3: Analgesic activity of *Tropaeolum majus* L in acetic acid induced test in mice

Groups	Dose (mg/kg, b.wt) i.p	No of writhing (10 min)
Control(saline water)	10ml/kg	66.8 ± 1.3277
Standard (Diclofenac sodium)	10	10.45 ± 0.1765**
Ethanolic extract	200	34 ± 1.820*
	400	29.75 ± 0.9376*
Aqueous extract	200	32 .50 ± 0.8466*
	400	28.66 ± 1.498*

Values are mean ± SEM (n=6) when compared with control * P<0.05, **P<0.01, ***P<0.001 were considered significant comparing to control

Anti-inflammatory activity:

Table 4: Anti-inflammatory activity of *Tropaeolum majus* L on paw edema induced by Carrageenan in rats

Groups	Dose mg/kg b.wt	0h	1h	2h	3h	4h	5h
Control (Saline water)	10 ml	0.62 ± 0.095	0.75± 0.164	0.96± 0.151	1.6 ± 0.300	1.7± 0.397	1.9 ± 0.528
Standard (Indomethacin)	10 mg	0.59 ± 0.003*	0.27 ± 0.024**	0.29 ± .007**	0.60±0.008***	0.44±0 .004**	0.48 ± 0.004**
Ethanolic extract	200	0. 58 ± 0.092^{ns}	0.70 ± 0.123^{ns}	0.82 ± 0.104*	0.75± 0.105^{ns}	0.90± 0.106*	0.95± 0.125^{ns}
	400	0.60 ± 0.146^{ns}	0.68± 0.120*	0. 80± 0.055*	0.70± 0.120**	0.82 ± 0.111*	0.97± 0.129*
Aqueous extract	200	0.56 ± 0.102^{ns}	0.66 ± 0.084**	0.77 ± 0.119*	0.68 ± 0.065**	0.79 ± 0.070^{ns}	0.93 ± 0.006*
	400	0.58 ± 0.050*	0.62 ± 0.100ns	0.75 ± 0.065*	0.64± 0.059*	0.77 ± 0.061**	0.90 ± 0.015^{ns}

Values are mean ± SEM (n=6) when compared with control * P<0.05, **P<0.01, ***P<0.001 were considered significant comparing to control

Table 5: Anti-inflammatory activity of *Tropaeolum majus* L on paw edema induced by histamine in rats

Groups	Dose mg/kg b.wt	0h	1h	2h	3h	4h	5h
Control (saline water)	10 ml	0.62 ± 0.095	0.75± 0.164	0.96± 0.151	1.6 ± 0.300	1.7± 0.397	1.9 ± 0.528
Standard (Indomethacin)	10 mg	0.59 ± 0.003*	0.27 ± .024*	0.29 ± 0.007**	0.60±0.008***	0.44± 0.004**	0.48 ± 0.004***
Ethanolic extract	200	0. 55±0.015^{ns}	0.69 ±0.124^{ns}	0.55 ± 0.102^{ns}	0.86±0. 092**	0.82± 0.091*	0.86± 0.094*
	400	0.56 ± 0.007*	0.59 ±0.042*	0. 52± 0.024*	0.83±0.023*	0.83 ± 0.144*	0.87±0 .052*
Aqueous extract	200	0. 60 ±0.049^{ns}	0.66± 0.047^{ns}	0.56 ± 0.035*	0.88± 0.700*	0.86± 0.632*	0.96±0 .118*
	400	0.59 ± 0.082^{ns}	0.60 ± 0.118^{ns}	0. 49± 0.044*	0.87± 0.128*	0.85± 0.079**	0.92±0 .122*

Values are mean ± SEM (n=6) when compared with control * P<0.05, **P<0.01, ***P<0.001 were considered significant comparing to control

Table 6: Anti-inflammatory activity of *Tropaeolum majus* L on cotton pellet induced granuloma in rats

Treatment	Dose (mg/kg)i.p	Weight of dry cotton pellet Granuloma(mg)
Control (saline water)	10ml/kg	208
Standard (Indomethacin)	10	13.9**
Ethanolic extract	200	130^{ns}
	400	98**
Aqueous extract	200	142^{ns}
	400	90**

Values are mean ± SEM (n=6) when compared with control * P<0.05, **P<0.01, ***P<0.001 were considered significant comparing to control

RESULTS

Acute toxicity studies. The leaf extract of *Tropaeolum majus* L were evaluated for acute toxicity in mice and rats by intraperitoneal and oral administration of extract. No mortality and behavioral changes were observed up to 2 weeks. The ethanolic & aqueous extract were safe up to 2000mg/kg body weight dose. Based on this test *Tropaeolum majus* L was tested at 200, 400 mg/kg body weight for this experiment.

Phytochemical Screening: Phytochemical screening of both ethanolic and aqueous extract showed the presence of flavonoids, tannins, glycosides, alkaloids, saponins & steroids.

Analgesic Activity

Tail immersion method in mice: Results of analgesic activity of *Tropaeolum majus* L ethanolic and aqueous leaf extract measured by tail immersion method are given in Table-1.At dose 200, 400 mg/kg *Tropaeolum majus* L extract exhibited 50 % inhibition when compared to control, whereas the positive control exhibited 97 % inhibition. From table-1, it is evident that both extract showed moderate analgesic activity when compared to that of diclofenac sodium.

***Hot plate method in rats*:** Results of analgesic activity of *Tropaeolum majus* L ethanolic and aqueous leaf extract measured by hot plate method are given in Table-2. The results of the hot plate test revealed that the most significant latency time was observed at dose 400 mg/kg for aqueous extract and the percentage inhibition was found to be 60.61%, when compared to ethanolic extract which was found to be 54.26%, whereas tramadol showed 87% inhibition when compared to control.

Acetic acid induced writhing test in mice: Results of analgesic activity of *Tropaeolum majus* L ethanolic and aqueous leaf extract are given in Table-3.Both ethanolic and aqueous extract showed a dose dependant decrease in abdominal constrictions in all the two doses in mice by using 1% acetic acid solution. The dose dependant protective effect reached a maximum inhibition of 55.46% for ethanolic extract and 57.09% for aqueous extract at dose 400mg/kg b.wt, diclofenac sodium (standard) exerted significant protective effect with percentage of protection 84.35% when compared to control.

Anti-inflammatory Activity:

Carrageenan- Induced paw edema in rats: Results of anti-inflammatory activity of *Tropaeolum majus* L ethanolic and aqueous leaf extract are given in Table-4.Injection of Carrageenan was done 1h after oral administration of the extract (200,400mg/kg b.wt), Indomethacin (reference drug) .Both the ethanolic and aqueous leaf extract showed significant inhibition of paw edema at 3h .Ethanolic extract showed 56.25%, whereas aqueous extract showed 64% at dose 400mg/kg when compared to control. Indomethacin showed inhibition of paw edema with a maximum effect of 78.25%.

***Histamine induced paw edema in rats*:** Results of anti-inflammatory activity of *Tropaeolum majus* L ethanolic and aqueous leaf extract are given in Table-5.Inflammatory edema induced by histamine was significantly inhibited in a dose dependant manner and significant inhibition of edema started at 3hr and significant up to 5^{th} hr.Ethanolic extract showed 54.73% inhibition, whereas aqueous extract showed 51.57% when compared to control. Indomethacin showed inhibition of paw edema with a maximum effect of 78.25%.

Cotton pellet granuloma in rats: Results of anti-inflammatory activity of *Tropaeolum majus* L ethanolic and aqueous leaf extract are given in Table-6.The leaf extract exhibited a significant and dose related inhibition of the dried weight of the cotton pellet granuloma. The inhibitory values for 200 and 400 mg/kg of ethanolic and aqueous extract exhibited 37.5%, 52.88%, 31.73%, 56.73% respectively. Indomethacin (reference drug) inhibited granuloma tissue formation with a value of 93.31%

DISCUSSION

In the present study analgesic activity of *Tropaeolum majus* L ethanolic and aqueous leaf extract were screened by three different methods (tail immersion, hot plate and writhing method). Anti-inflammatory activity was determined by three different methods (carrageenan, histamine induced paw edema & cotton pellet granuloma).Both the activities were determined at dose levels 200, 400 mg/kg b.wt. Diclofenac sodium, tramadol, Indomethacin was used as standard reference drugs. Central analgesic effects of drugs was determined by tail immersion method, analgesic effect through thermal noxious stimuli may be elicited through opioid receptors or through modulation of several neurotransmitters involved in relevant phenomenon. All the extract increased basal latency probably by acting through centrally mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain while nonsteroidal anti-inflammatory drugs inhibit only peripheral pain [19].

The analgesic effect of *Tropaeolum majus* L ethanolic and aqueous leaf extract was screened using eddy's hot plate method. This animal model shows marked central analgesic effect .Thermal test was selected because of several advantages including sensitivity to strong analgesics and limited tissue damage. All the extract showed significant latency time. Writhing test is a chemical method used to induce pain of peripheral origin by injection of irritant principles like phenylquinone or acetic acid in mice. Analgesic activity of the leaf extract is inferred from decrease in the frequency of writhes. The response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and prostaglandin pathways. Decrease in writhes is generally considered as an important parameter of analgesic activity in acetic acid induced writhing test [20].

Anti-inflammatory effect was evaluated in the acute phase of inflammation and chronic phase of inflammation. Carrageenan was selected because of its sensitivity in detecting orally acting anti-inflammatory agents in the acute phase of inflammation [21]. The cotton pellet granuloma method is a model of chronic inflammation and the dry weight has been shown to correlate with the amount of granulomatous tissue formed. Carrageenan induced edema is well established model and is believed to be biphasic. The initial phase has been known (1-2h) to be induced due to the action of mediators such as histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandins release and mediated by bradykinin, leukotrienes, polymorhonuclear cells and prostaglandins produced by tissue macrophages. All the extract showed significant inhibition of paw edema induced by carrageenan and histamine by inhibition of Cycloxygenase synthesis. The cotton pellet granuloma method has been widely used to evaluate transudative, exudative and proliferative components of chronic inflammation [22], because the dried weight of the pellets correlates with the amount of granulomatous tissue, all the extract showed dose-dependent inhibition of granuloma formation in mice.

CONCLUSION

The present study of *Tropaeolum majus* L ethanolic and aqueous leaf extract showed potent analgesic and anti-inflammatory activity. The activity may be due to the presence of chemical constituents mainly flavonoids, saponins that are present as chemical constituents in these extract. Flavanoids and saponins are well known for their ability to inhibit pain perception as well as anti-inflammatory properties due to their inhibitor effects on enzymes involved in the production of the chemical mediator of inflammation. The presence of flavonoids & saponins may be responsible for analgesic and anti-inflammatory activity, further investigation are required to isolate the active constituents and to know the possible mechanism of action of the plant extract.

REFERENCES

1. Fields HL, Martin JB, Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL. Pain pathophysiology and management In: Harrison's Principles of internal medicine (17th ed).New York; McGraw Hill: 2008, pp 81-86.
2. Anil Kumar M. "Ethnomedicinal plants as anti-inflammatory and analgesic agents" in Ethnomedicine: A Source of Complementary Therapeutics, Research signpost, 2010; 267-293.
3. Dhirender K, Ajay K, Pawan K and Rana AC. Analgesic and Anti-inflammatory Activity of *pinus roxburghii Sarg*.Advances in Pharmacological Sciences, 2012; 1-6.
4. Muhammad M, Mohammed R, Alhajhoj, Abdul AK, Jalal-ud-Din B. Flowering time response of Nasturtium (*Tropaeolum majus* L.) Cultivar "Empress of India" to photoperiod, light integral and temperature using photo-thermal model.Songklanakarin J.Sci.Technol, 2015; 37(3):245-254.
5. Brickell C.RHS Encyclopedia of plants and Flowers, Dorling Kindersley Publishers Ltd, Landon, UK. 2008.
6. Lens L and Birger LM. Synthesis of Benzylglucosinolate in *Tropaeolum majus* L. Plant Physiol, 1993, 102.609-613.
7. Zanetti GD, Manfron MP, Hoelzel SCS. Analise morfo-anatomica de *Tropaeolum majus* L. (Tropaeolaceae). Iheringia Serie Botanica, 2004; 59:173-178.
8. Arquimedes GJ, Francielly MG, Marcos AB, Emerson LBL, Maria EAS, Marcos JS, Jose EDSS, Maria CAM, Candida ALK. Journal of Ethnopharmacology, 2011; 134:210-15.
9. Arquimedes GJ, Francielly MG, Emerson LBL, Sandra C, Maria EAS, Marcos JS, Jose EDSS, Maria CAM, Candida ALK. Journal of Ethnopharmacology, 2011; 134:363-372.
10. Monica B and Cristian B. Antimicrobial and anti-inflammatory activities of the volatile oil compound from *Tropaeolum majus* L.(Nasturtium).African Journal of Biotechnology,2011;10(31):5900-5909.
11. Silva ME, Mussury RM, Vieira Mdo C, Alves Junior VV, Pereira ZV, Scalon SP. Floral biology of *Tropaeolum majus* L. and its relation with Astylus Variegatus activity. An Acad Bras Cienc, 2011;83(4):12451-8.
12. OECD/OCDE.OECD (423) guidelines for testing of chemicals. Acute oral toxicity up and down procedure, 2001:1-26.
13. Singh S, Majumdar D, Rehan H. Evaluation of anti-inflammatory potential of fixed oil of *ocimum sanctum*(Holybasil) and its possible mechanism of action. J.Ethnopharmacology, 1996; 54(1):19-26.
14. Kulkarni SK.Handbook of experimental pharmacology 9th edition Vallabah prakashan New Delhi: 2007, pp .125-127.
15. Koster R, Anderson M and De Beer J. "Acetic acid for analgesic screening". Federation proceedings, 1959; 18:pp.412-417.
16. Winter CA, Risley EA and Nus GW. "Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. " Proceedings of the society for Experimental Biology and Medicine, 1962; 111: pp.544-547.
17. Singh S, Majumdar D, Rehan H. Evaluation of anti-inflammatory potential of fixed oil of *Ocimum sanctum* (Holybasil) and its possible mechanism of action.J.Ethnopharmcol, 1996;54(1):19-26.
18. Winter CA and Porter C. "Effect of alterations in the side chain upon anti-inflammatory and liver glycogen activities of hydrocortisone esters" .Journal of the American Pharmaceutical Association, 1957; 46:515-519.
19. Elisabetsky E, Amador TA, Albuquerque RR, Nunes DS and Carvalho Ado C. Analgesic activity of *Psychotria colorata* (wild.ex R.&S.) Muell Arg. alkaloids. J.Ethanopharmacology, 1995; 48(2):77-83.
20. Ribeiro RA, Vale S, Thomazzi SM et al. " Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice". European Journal of Pharmacology, 2000; 387(1):111-118.
21. Gupta M, Mazumder UK, Gomathi P and Selvan VT. "Anti-inflammatory evaluation of leaves of plumeria *accuminata*". BMC complementary and Alternative Medicine, 2006; 6:1-6.
22. Swingle KF and Shideman FE. "Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their modification by certain anti-inflammatory agents". Journal of Pharmacology and Experimental Therapeutics, 1972;183(1).226-234.