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Research Article

# ANTIFUNGAL, ANTI-INFLAMMATORY AND GC – MS ANALYSIS FOR BIOACTIVE MOLECULES OF *TRIDAX PROCUMBENS L.* LEAF

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**ABSTRACT**

The traditional use of medicinal plants leaf extract for diseases is quite common in developing countries like India. *Tridax procumbens .L* is one such plant very commonly used for wound healing. In a view to understand the scientific reason behind its medicinal value, an attempt is made in this study, to analyze major bioactive compounds present in the essential oil extracted from *Tridax procumbens .L* leaf by GC-MS. The antifungal and anti-inflammatory activities of the methanolic extract of *Tridax procumbens .L* leaf were also investigated. The antifungal activity was assessed by Zone of Inhibition using the Minimal Fungicidal Concentrations of the extracts such as 150, 250, 500 µg/ml. The result reveals that the different concentration of the extract shows good antifungal activity when comparing with the positive control. For the anti-inflammatory study, the methanolic extract of *Tridax procumbens L*. was given intraperitoneally (i.p.,) to Balb/c mice in the form of suspension in 2% gum acacia in two different doses, 250 and 500 mg/kg. The anti-inflammatory effect of *Tridax procumbens L.* was tested in mice in inflammatory condition induced by different agents such as Carrageenan, egg-albumin, formalin and xylene. The result showed that the *Tridax procumbens L.* has significant reduction in inflammation in mice treated with both the concentration of extracts. However, 500mg/kg gives the faster reduction in the inflammation than the 250mg/kg does when compared with control and standard drug Diclofenac. This study indicate that the extract possess antifungal and anti-inflammatory properties. GC-MS was performed in essential oil of plant using the CARBOWAX capillary column and Helium as carrier gas and the result reveals the presence of α–pinene, β-pinene, Phellandrene, Sabinene as major bioactive compounds. Further study is required to find out the specific photochemical which is responsible for its medicinal value.

**Keywords:** Tridax procumbens, Diclofenac, GC – MS, anti – inflammatory, α–pinene, Sabinene.

## INTRODUCTION

World Health Organization (WHO) has estimated that more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases[1]. *Tridax procumbens* L. is a common grass found in the tropics. Traditionally, it is used for malaria, stomachache, high blood pressure, hemorrhage and to prevent hair fall as well. It possesses antiseptic, insecticidal, parasiticidal and hepatoprotective properties[2-4]. Humans and animals are prone to infection by several microorganisms, especially fungi[5, 6]. In general medicinal plants represent a rich source of antimicrobial agents[7]. Plant materials that are used in traditional medicines are readily available and are relatively cheaper than modern medicine[8]. Researchers are now in search of the effects of various plant extracts on bacteria[9, 10]. Reports are available on *in vitro* and *in vivo* efficacy of plant extracts against plants and human pathogens causing fungal infections[11]. Keeping this in view, the present study has been undertaken to evaluate the anti - fungal and anti-inflammatory effects of methanolic extract of *Tridax procumbens* L. leaf. Inflammation is a tissue reaction to infection, irritation or foreign substances. There are several tissue factors or mechanisms that are known to be involved in the inflammatory reaction such as release of histamine, bradykinin and prostaglandins. In addition to local changes in an inflammatory area, there are often various responses such as rise in temperature, increase in blood leucocytes etc. There is also an increase in certain plasma proteins termed acute phase proteins[12]. Histamine has been implicated as a mediator of vasodilatation and other changes that occur during inflammation. It promotes adhesion of leukocytes to vascular endothelium by expressing adhesion molecule P-selection on endothelial cell surface, sequestrating leukocytes at the inflammatory site. It may also regulate microcirculation according to the local needs[13]. All these events result in swelling at the site of inflammation. Generally, plants produce secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs[14]. Fewer reports are available with respect to the medicinal properties of the plant. It is essential to identify the bioactive molecules present in each medicinal plant responsible for its pharmacological effect. GC-MS is the best tool to study such bioactive compounds present in plant extracts. The analysis of phytochemicals presents in *Tridax procumbens* leaf using GC-MS is also a focus of this study.

## MATERIALS AND METHODS

### Plant Material

*Tridax procumbens* L. leaves was collected from Western Ghats of Siruvani hills of Coimbatore, India. The plant materials were taxonomically identified and authenticated by the Botanical Survey of India and the voucher specimen (**No.BSI/SC/5/23/09-10/TECH.1448**) was retained in our laboratory for future reference.

### Preparation of plant Extract

The plant was freshly collected to about 5kg and were shade dried until all the water molecules were evaporated (15 -30 days). After drying, the plant leaves were ground well using mechanical blender into fine powder and then transferred into airtight containers for future studies. The fine powder (of about 100grams in 1000ml of methanol i.e., 1:10 ratio) is then subjected to soxhlet apparatus for the extraction of pure form of the plant leaf extract. The extract was filtered and the filtrate was concentrated at 30ºC under reduced pressure in a rotary evaporator. The yield (w/w) of the crude extract was found to be 12.06%. The crude extract was then dissolved in methanol and when used, the methanol was evaporated and used for further experiments.

### ANTI-FUNGAL ACTIVITY STUDY

### Fungal inoculum preparation

The fungal cultures *Candida albicans, Aspergillus fumigates, Candida tropicalis, A. flavus and A. niger were* used for the study. These fungal cultures were maintained in potato dextrose agar plates and slants, which were further sub cultured before use. The mother inoculum was maintained at $37^0$C for about 48 to 72 hours. The fungal spores were scooped out by adding 1ml of sterile distilled water. The fungal spores were collected to about 1 ml and it was serially diluted from $10^{-1}$ to $10^{-6}$ and plating was done using $10^{-4}$ dilution.

### Determination of Minimum Fungicidal Concentration (MFC)

MFC was determined by agar dilution method. Various concentrations (50, 100, 150, 200, 250, 300, 350, 400, 450, 500 µg/ml) of each extracts were prepared in 10 cm experimental tubes containing PDA broth. Each tube contains 9 ml of PDA and was sterilized by autoclaving. Upon cooling, 1 ml of extract was added.

The mixture PDA and extracts were poured into plates aseptically in a laminar flow cabinet. Upon solidification of the agar medium, 2µl of adjusted spore suspension were added to each plate by micropipette and incubated at 28° C for 3 days. The PDA without any herbal extract served as negative control.

**Agar Well diffusion method for anti-fungal activity study**

The solidified agar plates were taken and divided into 3 quadrants. Each quadrant is marked as 500µg, 250µg, 125µg. Negative control and Positive control were maintained separately. The inoculum from $10^{-4}$ dilution was taken to spread the fungal spores on the potato dextrose agar plate. 100µl of plant extracts of each concentration was transferred into respective wells as per the markings and 100µl of methanol was added to the negative control well. In the positive control Fluconozole anti fungal disc was placed for the comparison of result. Three replicates were used per treatment. The plates were kept for incubation at 37 ºC to about 48 to 72hrs. After 72 hrs of incubation the ZOI was clearly visible and the zones were measured.

**Experimental animals for anti-inflammatory study**

BALB/c (25-30g), were used for the study (6/group/cage) and maintained under temperature 24-28 ºC, RH – 60-70% and 12 hours light and dark cycles. Mice were housed in cages for at least one week before starting experiments and were fed with commercial mice feed (Sri Sai Durga Feeds and Food, Bangalore) and boiled water, ad libitum. All the experiments involving animals were performed according to the standard protocols from NIN guidelines, after getting proper approval.

**Acute toxicity study**

Overnight-fasted BALB/c (25-30g) of either sex was used. Animals were divided into 5 groups of 5 animals each. Groups A to D received orally 50, 150, 250 and 500mg/kg of the extract, respectively, while the control i.e., Group E - received distilled water (3 ml/kg) by the same route. General symptoms of toxicity and mortality in each group were observed within 24 h. Animals that survived after 24 h were observed for any signs of delayed toxicity for two weeks. For the further study 250 and 500mg/kg doses were selected.

**ANTI-INFLAMMATORY ACTIVITY STUDY**

**Xylene induced ear inflammation**

BALB/c (25-30g) was divided into four groups (6 / Group). Animals were treated Intra peritoneally with the extract (250 and 500 mg) to group 3 and 4, Diclofenac (100 mg/kg) to group 2 and 0.1ml of 2% gum acacia to Group 1. Thirty minutes later, inflammation was induced in each mouse group by placing a drop of xylene to the inner surface of the right ear. After 15 min, the animals were sacrificed under ether anesthesia and ears were cut off, sized and weighed[15]. The anti-inflammatory activity was expressed as the % inhibition of inflammation in the treated mice in comparison with the control mice.

**Carrageenan - Induced paw inflammation in Mice**

Anti-inflammatory activity of *Tridax procumbens* L. was assessed by Carrageenan induced paw inflammation method. Mice were divided into 4 groups (6 animals / group) [30]. Animals of all the groups were injected with 0.1 ml of 1% Carrageenan in 0.9% saline, under the foot pad aponeurosis of the right hind paw. Group I animals (Carrageenan control) received 0.1ml of 2% gum acacia i.p., 30 min before Carrageenan injection. Group II, was given the standard drug Diclofenac (100 mg) 30 min before Carrageenan injection. Group III and Group IV received i.p., the different concentration such as 250 mg/kg and 500 mg/kg of *Tridax procumbens* L. methanolic extract suspension in 2% gum acacia, 30 min prior to Carrageenan injection, respectively. The paw volume of the mice was measured using Vernier caliper prior to and after ($0^{th}$ hour, every 30min between $1^{st}$ - $8^{th}$ hour, $12^{th}$ and $24^{th}$ hour) Carrageenan injection.

**Egg - albumin- induced inflammation in Mice**

BALB/c (25-30g of either sex randomized into 4 different groups of 6 mice each were used for the experiment. The leaf extract with a concentration of 250 and 500 mg and Diclofenac (100mg orally) were administered to mice 1 hr before the induction of inflammation. Negative control group received 0.1ml of 2% gum acacia i.p. Inflammation was induced by 0.1 ml of fresh egg-albumin into the sub planar tissue of the right hind paw. The Inflammation was measured before and after 30 min and again from $1^{st}$ to $5^{th}$ hour after the administration of the phlogistic agent[16]. The inflammation was assessed by measuring with Vernier caliper.

**Formalin induced inflammation in Mice**

Anti-inflammatory activity was evaluated by formalin induced paw inflammation method. BALB/c 25-30g of either sex was divided into 4 groups (6 animals / group). Animals of all groups were administered with 0.1 ml of 1% formalin in 0.9% saline, under the plantar aponeurosis of the right paw. Group I animals (formalin control) received 0.1ml of 2% gum acacia i.p. 30 min before formalin injection, Group II was given i.p., standard drug Diclofenac (100 mg) 30 min prior to formalin injection. Group III and Group IV received i.p., 250 and 500 mg/kg of *Tridax procumbens* L. Methanolic extract suspension in 2% gum acacia, 30 min prior to formalin injection. The paw volume of the mice was measured using Vernier caliper just before and after 3rd, $6^{th}$, $12^{th}$ and $24^{th}$ hour following formalin injection. The percentage inhibition of the inflammation was calculated and compared with the control group[17].

**GC-MS analysis**

GC-MS analysis was performed in INDIAN INSTITUTE OF SPICES RESEARCH (IISR)-CALICUT-KERALA- [PMT/IISR/28(13)09]. Essential oils were extracted from *Tridax procumbens .L*, based on hydro distillation method. GC-MS analysis was performed using CARBOWAX capillary column and Helium as carrier gas to quantify the major phytochemicals present in essential oil. 0.2µl of essential oil was injected in to the column of 1µl/min at $250^0$C and the oven temperature was programmed as $60^0$C for 15minutes, and then gradually increased to $280^0$C for 3minutes. The identification were based on comparison of their mass spectra and retention indices.

**Statistical analysis**

Data was statistically analyzed using one – way ANOVA as primary test followed by Dennett's test using Graph pad InStat3.0 software for Windows XP, Graph pad Software, San Diego, California, USA.

**RESULT AND DISCUSSION**

Several plants are used traditionally as medicinal agent for internal and tropical application. The very common plant used for injury is *Tridax procumbens .L* as extract. However, the scientific validation on its medicinal value may help to develop better drugs formulations. In this study, pharmacological evaluation of antifungal and different anti-inflammatory activities of Methanolic extract of *Tridax procumbens. L* was carried out using different experimental models. Scientifically it is necessary to investigate plants that have been used in traditional medicines to determine their potential sources of novel antimicrobial compounds[18].

**Table 1: Minimum fungicidal concentration (MFC) of *T.P.L* on selected fungal species**

| Fungal species | Concentration of plant extract (µg/ml) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50 | 100 | 150 | 200 | 250 | 300 | 350 | 400 | 450 | 500 | control |
| *Candida albicans* | - | + | + | + | + | + | + | + | + | + | - |
| *Aspergillus fumigatus* | + | + | + | + | + | + | + | + | + | + | - |
| *Candida tropicalis* | + | - | + | + | + | + | + | - | + | + | - |
| *Aspergillus flavus* | - | - | + | - | + | - | - | - | - | + | - |
| *Aspergillus niger* | - | - | - | - | - | - | - | + | - | + | - |

**Table 2: Zone of Inhibition (ZOI) of *T.P.L* on selected fungal species.**

| S.NO | Cultures and Dilution used($10^{-4}$) | Zone of inhibition (cm) of *Tridax procumbens. L* 150 µg/ml | 250 µg/ml | 500 µg/ml | Positive control | Negative control |
|---|---|---|---|---|---|---|
| 1 | *Candida albicans* | 14±1.00 | 14.3±0.57 | 15±1.00 | 23.6±1.52 | 22±3.60 |
| 2 | *Aspergillus fumigatus* | 12.3±0.57 | 14±0.00 | 16.6±0.57 | 22±1.00 | 21.3±3.05 |
| 3 | *Candida tropicalis* | 12.3±0.57 | 13.3±0.57 | 14.3±0.57 | 23±1.00 | 20±1.73 |
| 4 | *Aspergillus flavus* | 13.3±0.57 | 14.6±1.15 | 15±1.00 | 20.3±1.52 | 21±2.64 |
| 5 | *Aspergillus niger* | 13.3±1.528 | 14.3±2.51 | 15.3±1.52 | 22.3±1.52 | 20.6±1.52 |

**Table 3: Effect of *T.P.L* leaf extract on Carrageenan Induced inflammation in mice**

| TREATMENT DOSE (mg/kg) | TIME INTERVALS (IN HOURS) READINGS (In cm) (In %) 0 | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 | 3.5 | 4 | 4.5 |
|---|---|---|---|---|---|---|---|---|---|---|
| CONTROL (10ml/kg) | 0.22 ±0.01 | 0.45(51) ±0.02 | 0.42(48) ±0.02 | 0.41(46) ±0.02 | 0.41(46) ±0.02 | 0.41(46) ±0.01 | 0.41(46) ±0.02 | 0.40(45) ±0.01 | 0.40(45) ±0.01 | 0.40(45) ±0.01 |
| STANDARD DRUG (100mg/kg) | 0.25 ±0.01 | 0.41(39) ±0.02 | 0.41(39) ±0.01 | 0.41(39) ±0.02 | 0.40(36) ±0.02 | 0.39(35) ±0.01 | 0.38(34) ±0.02 | 0.37(32) ±0.01 | 0.37(32) ±0.01 | 0.36(30) ±0.02 |
| EXTRACT (250 mg/kg) | 0.23 ±0.01 | 0.43(46) ±0.01 | 0.42(45) ±0.01 | 0.42(45) ±0.01 | 0.41(44) ±0.01 | 0.39(41) ±0.01 | 0.38(39) ±0.01 | 0.36(36) ±0.01 | 0.35(34) ±0.01 | 0.34(32) ±0.01 |
| EXTRACT (500mg/kg) | 0.24 ±0.01 | 0.42(43) ±0.01 | 0.42(42) ±0.01 | 0.42(42) ±0.01 | 0.40(40) ±0.01 | 0.40(40) ±0.01 | 0.39(38) ±0.01 | 0.38(36) ±0.01 | 0.38(36) ±0.01 | 0.37(35) ±0.01 |

| TREATMENT DOSE (mg/kg) | TIME INTERVALS(IN HOURS) READINGS (In cm) (In%) 5 | 5.5 | 6 | 6.5 | 7 | 7.5 | 8 | 12 | 24 |
|---|---|---|---|---|---|---|---|---|---|
| CONTROL (10ml/kg) | 0.39(44) ±0.01 | 0.38(42) ±0.01 | 0.35(37) ±0.01 | 0.34(37) ±0.01 | 0.34(37) ±0.01 | 0.33(33) ±0.01 | 0.32(30) ±0.01 | 0.31(29) ±0.01 | 0.31(29) ±0.01 |
| STANDARD DRUG (100mg/kg) | 0.35(28) ±0.02 | 0.34(26) ±0.01 | 0.33(24) ±0.01 | 0.32(22) ±0.01 | 0.32(22) ±0.01 | 0.31(19) ±0.01 | 0.30(16) ±0.02 | 0.29(13) ±0.01 | 0.27(7) ±0.01 |
| EXTRACT (250 mg/kg) | 0.33(30) ±0.01 | 0.32(28) ±0.01 | 0.31(26) ±0.01 | 0.30(23) ±0.01 | 0.29(21) ±0.01 | 0.28(18) ±0.01 | 0.28(18) ±0.01 | 0.28(18) ±0.01 | 0.28(18) ±0.01 |
| EXTRACT (500mg/kg) | 0.36(33) ±0.01 | 0.35(31) ±0.01 | 0.34(29) ±0.01 | 0.33(27) ±0.01 | 0.33(27) ±0.01 | 0.30(20) ±0.02 | 0.29(17) ±0.02 | 0.28(14) ±0.01 | 0.27(11) ±0.01 |

**Table 4: Effect of *T.P.L* leaf extract on Xylene Induced inflammation in mice**

| TREATMENT DOSE (mg/kg) | Weight of Right ear (g) | Weight of Left ear (g) | Increase in ear weight (g) | % Increase in ear weight | % Inhibition |
|---|---|---|---|---|---|
| CONTROL (10ml/kg) | 0.123 ±0.001 | 0.058 ±0.001 | 0.069 ±0.001 | 56.09 | - |
| STANDARD DRUG (100mg/kg) | 0.111 ±0.001 | 0.055 ±0.001 | 0.056 ±0.001 | 50.45 | 89.92 |
| EXTRACT (250 mg/kg) | 0.108 ±0.003 | 0.052 ±0.004 | 0.056 ±0.002 | 51.85 | 92.44 |
| EXTRACT (500mg/kg) | 0.103 ±0.002 | 0.048 ±0.004 | 0.055 ±0.002 | 53.39 | 95.18 |

**Table 5: Effect of *Tridax procumbens* L Leaf extract on Formalin Induced inflammation in mice**

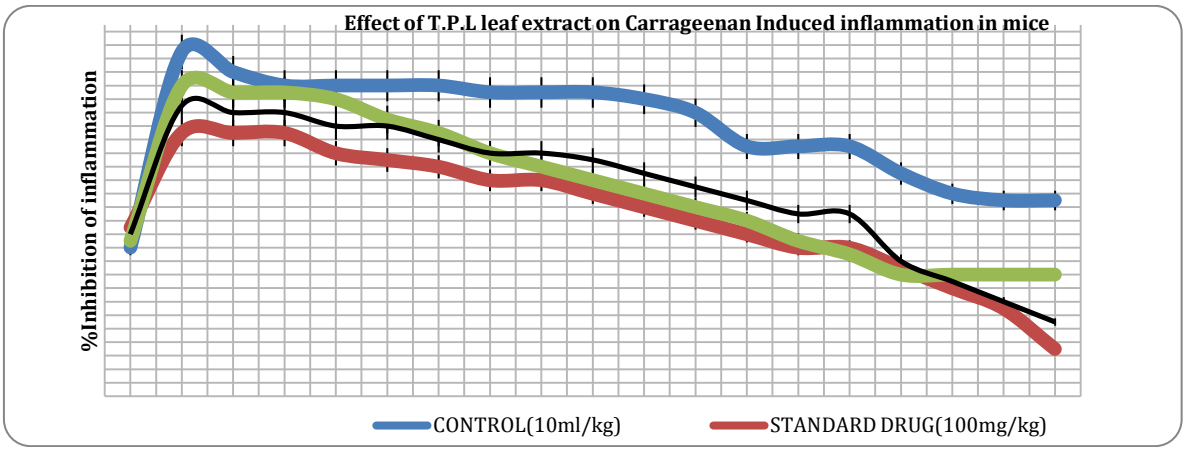
| TREATMENT DOSE (mg/kg) | TIME INTERVALS (IN HOURS) READINGS (In cm) (In %) Initial 0th Hour | 1st hour | 3rd hour | 6th hour | 9th hour | 12th hour | 24th hour |
|---|---|---|---|---|---|---|---|
| CONTROL (10ml/kg) | 0.34 ±0.01 | 0.54(37) ±0.01 | 0.47(27) ±0.007 | 0.45(20) ±0.008 | 0.43(20) ±0.01 | 0.42(19) ±0.01 | 0.40(15) ±0.03 |
| STANDARD DRUG (100mg/kg) | 0.32 ±0.009 | 0.52(38) ±0.01 | 0.44(27) ±0.007 | 0.42(23) ±0.01 | 0.41(21) ±0.01 | 0.40(20) ±0.05 | 0.37(13) ±0.01 |
| EXTRACT (250 mg/kg) | 0.33 ±0.01 | 0.53(37) ±0.01 | 0.45(26) ±0.01 | 0.45(26) ±0.01 | 0.42(21) ±0.01 | 0.41(19) ±0.006 | 0.38(13) ±0.008 |
| EXTRACT (500mg/kg) | 0.32 ±0.009 | 0.53(39) ±0.01 | 0.44(27) ±0.01 | 0.42(23) ±0.02 | 0.41(21) ±0.02 | 0.40(20) ±0.005 | 0.34(5) ±0.003 |

**Table 6: Effect of *T.P.L* leaf extract on Egg- Albumin Induced inflammation in mice**

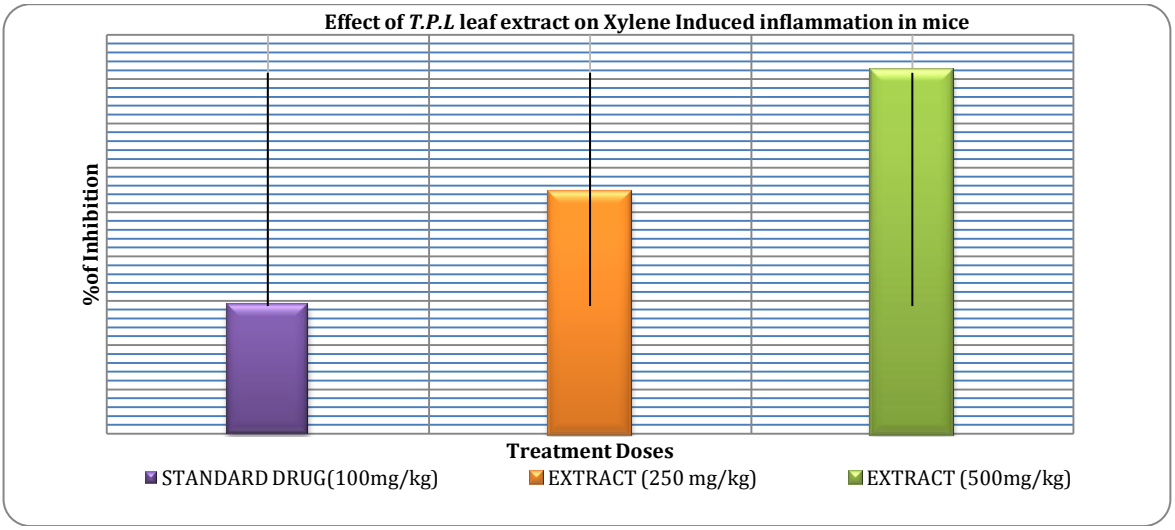
| TREATMENT DOSE (mg/kg) | TIME INTERVALS (IN HOURS) READINGS (In cm) (In %) Initial 0th Hour | 1st Hour | 2nd Hour | 3rd Hour | 4th Hour | 5th Hour |
|---|---|---|---|---|---|---|
| CONTROL (10ml/kg) | 0.34 ±0.005 | 0.48(29) ±0.008 | 0.43(21) ±0.01 | 0.42(19) ±0.01 | 0.41(17) ±0.006 | 0.40(15) ±0.004 |
| STANDARD DRUG (100mg/kg) | 0.31 ±0.005 | 0.45(31) ±0.005 | 0.42(26) ±0.008 | 0.40(22) ±0.007 | 0.38(18) ±0.008 | 0.36(13) ±0.01 |
| EXTRACT (250 mg/kg) | 0.31 ±0.005 | 0.44(29) ±0.008 | 0.41(24) ±0.005 | 0.38(18) ±0.005 | 0.35(11) ±0.007 | 0.34(8) ±0.007 |
| EXTRACT (500mg/kg) | 0.32 ±0.005 | 0.44(27) ±0.008 | 0.40(20) ±0.02 | 0.36(11) ±0.01 | 0.34(5) ±0.006 | 0.34(5) ±0.005 |

**Table 7: GC – MS ANALYSIS of *TRIDAX PROCUMBENS. L***

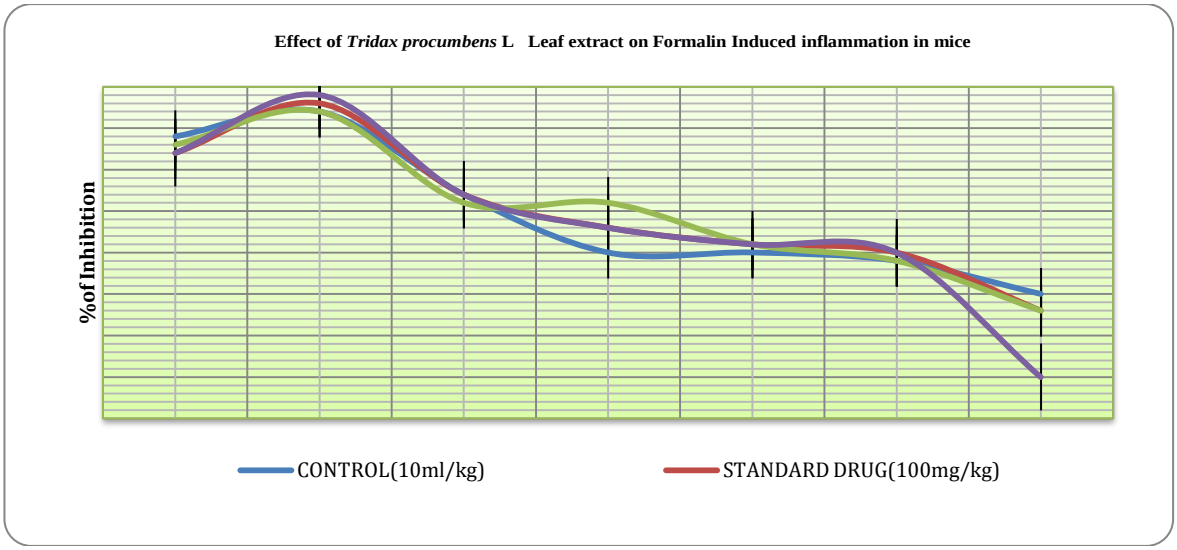
| S.NO | NAME OF THE PLANT | COMPOUNDS IDENTIFIED (IN NOs) | MAJOR COMPOUNDS |
|---|---|---|---|
| 1. | ***Tridax procumbens L.*** | 15 | α -pinene, β-pinene, l- phellandrene, Sabinene – 7% |



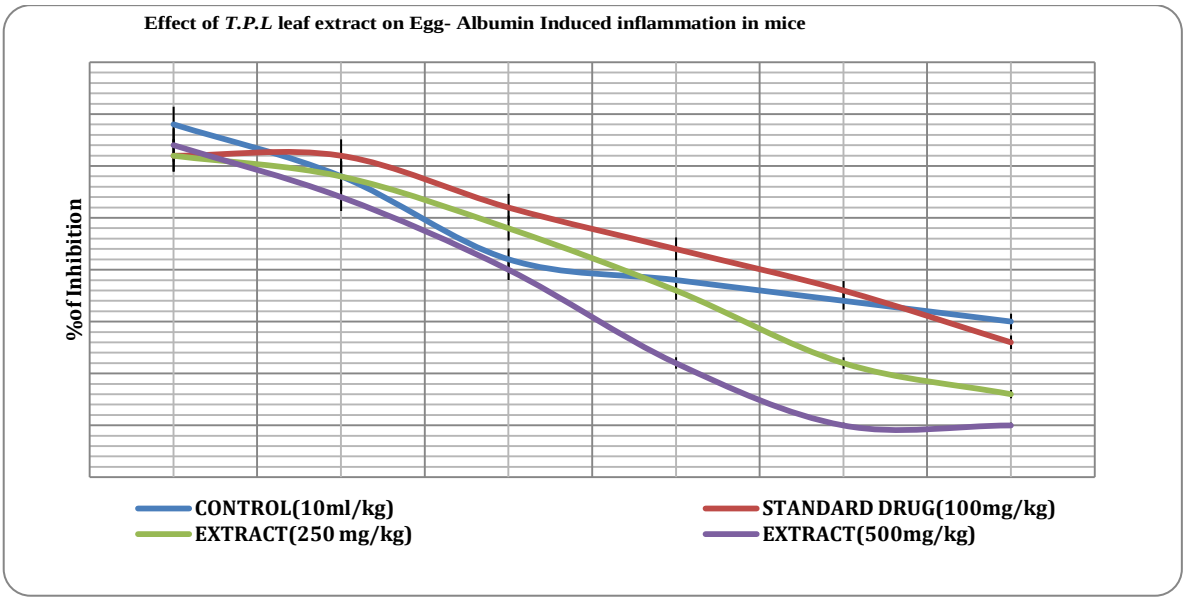

**Graph: 1**



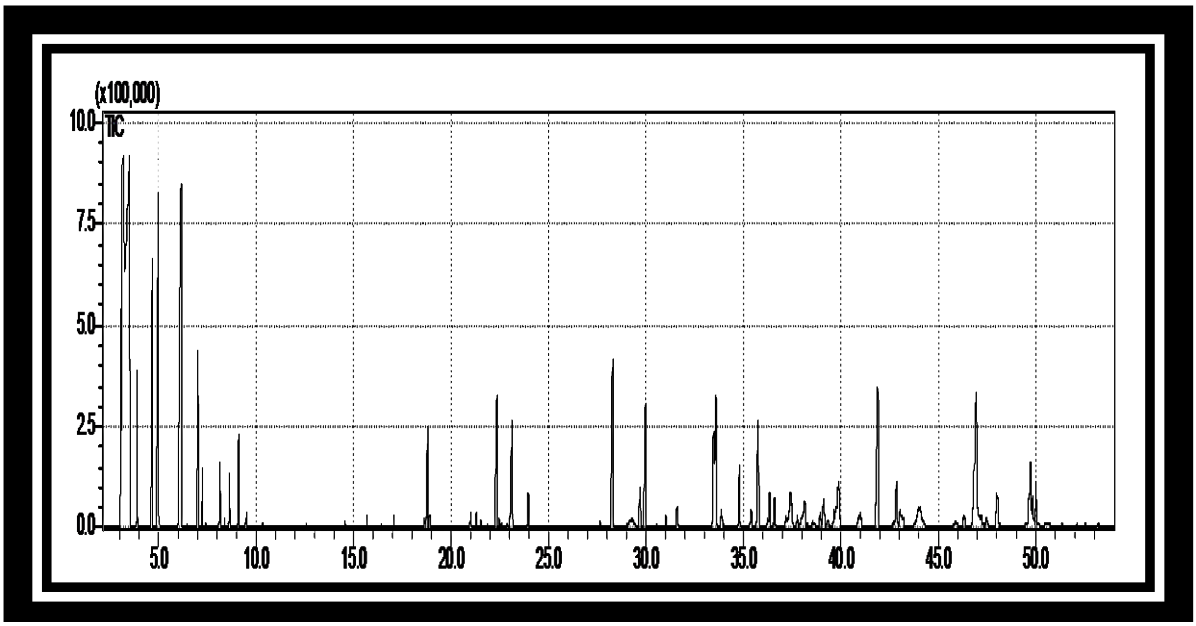

**Graph: 2**




**Graph: 3**




**Graph: 4**




**Graph 5: GC-MS Analysis Of *T.P.L* Leaves**

*Tridax procumbens. L* leaf extract at different concentration of 150, 250, and 500µg/ml possess an inhibitory effect against the fungal species taken for the study. However, our findings indicate that all the tested fungal species were susceptible and some are resistant to the standard anti fungal disc Fluconozole. Use of such natural fungicidal agents may be effective and less toxic than the commercial chemical fungicides available. These mechanisms that is responsible for the antifungal activity is thought to be because of the phytochemicals present in the plant that shows a greater inhibitory activity against microorganisms[19]. These mechanisms of actions include enzyme inhibition by the oxidized compounds, and act as a source of stable free radical leading to the inactivation of the protein and loss of function. It also processes the ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls and disrupts microbial membranes[20], some of them have the ability to intercalate with DNA, formation of ion channels in the microbial membrane, competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors[21].

Widely used test to scrutinize the new anti-inflammatory substances is by measuring the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent. Carrageenan induced inflammation has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. Carrageenan is a sulphated polysaccharide obtained from seaweed, which is widely used as phlogistic agent which shows signs and symptoms of inflammation and can be assessed from an increase in the paw thickness, which result in the increased inflammation and increased vascular permeation. There are three phases of inflammation reported in which the early phase (1 – 2 h) of the Carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings cause edema and redness. The late phase is sustained

by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages and different cytokines and kinnins get released in response to the inflammation and the secreted mediators at the localized site.

In the third phase, the COX enzyme plays pivotal role leading to the production of prostaglandins which induces pain[22]. In the present study, *Tridax procumbens. L* leaf extract showed inhibition of paw thickness at 3rd – 5th hour and 7th -24th hours which probably suggests that it inhibits the prostaglandin formation in the third phase of inflammation and other mediators in first two phases. The extract was effective against formaldehyde and egg albumin induced oedema, where it may inhibits inflammation by blocking the release of histamine and 5-HT, two mediators that are released by egg albumin. The leaf extract exerted a significant inhibition of ear inflammation caused by xylene only at the highest dose of the extract (500mg). This suggests the inhibition of phospholipase $A_2$ which is involved in the pathophysiology of inflammation due to xylene. The experimental evidence obtained indicates that the extract reduced formalin induced paw inflammation in mice.

The major bioactive compounds identified in our GC-MS study of the essential oil obtained are alpha (α) and beta (β) pinenes, Sabinene, and l-Phellandrene have tremendous medicinal value. α and β pinenes are volatile components of turpentine they are the dominant odorous compounds emitted by trees, shrubs, flowers and grasses and α-pinene and Sabinene are used against mushrooms and yeasts (dermatophytes)[23]. The effects of α-pinenes vary depending on the composition of monoterpenes and sesquiterpenes. However, a study reports that the antibacterial effect of these terpenes works out with both the Gram-negative and Gram-positive bacteria as well it is mentioned as a strong antifungal activity[24].

These compounds also possess anti-inflammatory properties[25]. Some specific studies show that β-pinenes, along with α-pinenes and other terpenes, are cytotoxic on cancer cells[26]. The β-pinenes also show antifungal properties[27,] especially on *Candida* spp[28]. Inhibition of mitochondrial respiration occurs when acting on yeast, in which it is found to, the proton pump activity and K+ transport, and to increase membrane fluidity[29].

## CONCLUSION

To conclude the study, the extract has demonstrated good antifungal and significant anti-inflammatory activity. These findings confirm its traditional medicinal use in the treatment of several inflammatory and painful conditions. The study reveals that both the antifungal and anti-inflammatory activity of the plant extract is dose dependent. The GC-MS analysis on this plant leaf extract showed the presence of important bioactive compounds such as α -pinene, β-pinene, l- phellandrene, Sabinene which found to have effect on antimicrobial and anti-inflammatory activity. Further, study is required to find out the accurate compound responsible for the plant's medicinal value. Moreover comparative analysis of medicinal plants phytochemicals gives a vast idea about the plants nature and their medicinal value from its essence of traditional usage.

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