Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 3, 2012

Research Article

EVALUATION OF ESSENTIAL OIL OF *TRIDAX PROCUMBENS L.* FOR ANTI-MICROBIAL AND ANTI-INFLAMMATORY ACTIVITY

A.MANJAMALAI[1], SRUTHI VALAVIL [2] AND V.M. BERLIN GRACE*

Department of Biotechnology, School of Biotechnology and Health Sciences, Karunya University, Coimbatore, Tamilnadu, India.
Email: berlsdg@gmail.com, berlin@karunya.edu, manja.malai58@gmail.com

Received: 14 Jan 2012, Revised and Accepted: 28 Feb 2012

ABSTRACT

The present Study is focused on the evaluation of the Anti-Microbial and Anti-Inflammatory activity of extracted essential oil of *Tridax procumbens* in the experimental animal models. The essential oil of *Tridax procumbens* was extracted and produced by Hydro distillation process by using water as solvent at its boiling point. The Anti-Microbial activity was evaluated by Disc Diffusion Method, using both fungal and bacterial strains. A minimum concentration of 50µg/ml of essential oil was used after determining the Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) of the essential oil and compared with the respective Positive and Negative controls. Maximum zone of inhibition was obtained for E. *coli, staphylococcus aureus, pseudomonas aeruginosa* as 16mm whereas for *streptococcus pneumonia* it was found to be 15.3mm. The Anti-inflammatory activity of the essential oil was determined in an inducing inflammation using inflammatory agents like Carrageenan, Egg-albumin and Xylene in the respective inflammatory sites and the results were compared with the control and standard anti-inflammatory drug, Diclofenac Sodium. The essential oil shows more or less equal activity with the standard drug with a decreased percentage of inflammation from 62%-16% (Carrageenan), 62%-16%(egg albumin) for standard drug and 62%-16% (Carrageenan), 62%-15% (egg albumin). The major bioactive compounds present in the essential oil of *Tridax procumbens* were identified using GC-MS analysis which includes alpha – pinene, Beta – Pinene, L – Phellandrene and Sabinene.

Keywords: *Tridax procumbens*, Anti-bacterial, Anti-fungal, Anti-inflammatory, MIC, MFC.

INTRODUCTION

The demand on plant based therapeutics seems to be increasing in both developing and developed countries due to the growing recognition that they are natural products, being non narcotic, having no side effects, easily available at affordable prices and sometime the only source of health care available to the poor. Medicinal plant sector has traditionally occupied an important position in the socio cultural, spiritual and medicinal arena of rural and tribal lives of India.

The major areas of drug from medicinal plants includes disease conditions for which modern drugs are either unavailable or unsatisfactory.[1] *Tridax procumbens* is a common annual weed in the west Africa sub region and tropical zones of the world like India and is known as "Coat Buttons". Traditional medicine practitioners (TMPs) and Tribal peoples of these areas use the leaves of the plant as a remedy against several ailments ranging conjunctivitis, diarrhoea and dysentery to wound healing and related inflammatory conditions.[2]

Various studies have been carried out using plant extract of *Tridax procumbens* in different solvents extraction such as total phenolics and antioxidant activity[3], Pharmacological evaluation[4, 5], Chemical profile[6], Effect on Lipo polysaccharide – induced hepatitis[7]. Essential oil is so termed as they are believed to represent the very essence of odor and flavor[8]. There is about three hundred essential oil in general use today by professional practitioners.[9]

Continuous damage has been encountered by viral, bacterial, parasitic and fungal contamination in our body. Essential oil plays a major role in protecting our body from this onslaught of pathogens, since immune system needs support and this essential oil can give the required endorsement[10]. Essential oil is also found to have wide use in pharmaceutical industries in development of drugs.[11]

In this study, our focus is to evaluate the role of bioactive molecule present in the essential oil of *Tridax procumbens* fresh leaves against bacterial and fungal species identified and isolated from the cancer patients. We have also studied the role of essential oil of *Tridax procumbens* in reducing the inflammation using in-vivo rat model (Swiss albino rats) by inducing inflammation with different inflammatory agents.

MATERIALS AND METHODS

Plant Material Collection and Authentication of plants

Fresh leaves of the selected plant *Tridax procumbens. L* having medicinal value 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.1449**) was retained in our laboratory for future reference.

Extraction of essential oil from study plant material

Extraction of essential oil from *Tridax procumbens* is done by Hydro distillation method[12] using Clevenger-type apparatus for 3 hours. Plant material (leaves) was immersed directly in a round bottom flask filled with water. This was then brought to boil. Vapours were condensed on a cold surface using condenser attached to it. Essential oil separation was based on the difference in density and immiscibility, which was then collected and dried over anhydrous sodium sulphate and stored in vial at low temperature until analysis.

Anti-Microbial Activity

Preparation of Inoculum

The bacterial and fungal cultures were obtained from Dr. N. G. P. Arts and Science College and Tamilnadu Agriculture University (TNAU), Coimbatore, Tamil Nadu. The bacterial cultures obtained are *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumonia* and the fungal cultures obtained are *Candida albicans, Candida tropicalis and Candida parapsilosis.* The bacterial cultures were maintained in Nutrient broth and Nutrient agar slants. The fungal cultures were maintained in Potato dextrose agar plates and slants. It was further sub cultured before use. The mother inoculum was maintained at ^{0}C37for about 24 hours (bacteria) and 48 to 72 hours (fungi). The bacterial and fungal strains were scooped out by adding sterile distilled water. The fungal and bacterial strains were collected to about 1 ml and it was serially diluted from 10^{-1} to 10^{-6} and plating was made using 10^{-4} dilution for fungal and 10^{-6} for bacterial inoculum.

Determination of Minimal Inhibition Concentration (MIC)

The minimal inhibition concentration (MIC) values were determined for the bacterial strains to the essential oils of *Tridax procumbens.* A

100µl of the inoculum, initially adjusted to 10^{-6} CFU/ml, was spread onto 20ml Muller–Hinton agar supplemented with the oil at concentrations ranging from 25, 50, 75 and 100 µg/ml in Petri dishes, with each one its equivalent in 50% Ethanol. These serially diluted cultures were then incubated at 37 ± 1°C for 24 h. The Lowest concentration inhibiting visible growth of test organism was observed and noted as the minimum inhibitory concentration (MIC). As control, 50% Ethanol was used. Tests were carried out in triplicate.[13]

Determination of Minimum Fungicidal Concentration (MFC)

The minimal fungicidal concentration (MFC) values were determined for the fungal strains to the essential oil of *Tridax procumbens.* The MFC was determined by incorporating various concentration of essential oil ranging from 25, 50, 75 and 100µg/ml in potato dextrose agar (PDA) tubes. One milliliter adjusted spore suspension was added to each tube and incubated at 28^{0}C for 3 days. The potato dextrose broth without incorporation of essential oil and 1 ml of adjusted spore suspension served as positive control and PDA broth alone served as negative control. The tubes, which showed no visible growth after three days of incubation, were sub cultured on extract free PDA plates and incubated at 28^{0}C for 3 days. Tests were carried out in triplicate.[14]

Antibacterial Screening

Disc diffusion method

The disc diffusion method[15] was employed for the determination of antibacterial activities of the essential oil. Paper discs (6 mm diameter) were impregnated with 50µg/ml of the oil dissolved in 50% Ethanol to a final concentration of 10% (v/v) and transferred onto the Nutrient Agar present in Petri dishes, which had been surface spread with 0.1 ml of bacterial suspension adjusted to 10^{-6} CFU/ml for all selected bacteria (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumonia*). The 50% Ethanol solvent was used as negative control and Standard antibiotic discs like Norfloxacin 10mcg, Cefepime 30mcg and Gatifloxacin 5mcg were used as positive controls. After incubation at 37 ± 1°C during 24 h, the diameters of inhibition zones were measured in millimeters. Tests were carried out in triplicates.

Antifungal Screening

Disc diffusion method

The disc diffusion method was employed for the determination of antifungal activities of the essential oil.[16] Paper discs (6 mm diameter) were impregnated with 50 µg/ml of the oil dissolved in 50% of Ethanol to a final concentration of 10% (v/v) and transferred onto the Potato Dextrose Agar present in Petri dishes, which had been surface spread with 0.1 ml of fungal suspension adjusted to 10^{-4} CFU/ml for selected fungal species (*Candida albicans, Candida parapsilosis, Candida tropicalis*). PDA plates, without essential oil were used as negative control and the one treated with antibiotic disc (Fluconozole 25mcg) was used as positive control. After incubation at 25 ± 2°C during 48 h, the diameters of inhibition zones were measured in millimeters. Tests were carried out in triplicates.

Anti-Inflammatory Activity

Experimental animals

Swiss Albino Rats (250-300g) were used for the study (5animals/group/cage) and were maintained under temperature 24-28 ºC, RH – 60-70% and 12 hours light and dark cycles. Rats 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 with boiled water. All the experiments involving animals were performed according to the standard protocols from NIN guidelines, after getting proper approval.

Acute toxicity study

Overnight-fasted Swiss Albino Rats (250-300g) of either sex was used. Animals were divided into 5 groups of 3 animals each. Each group of animals was given different doses of drug ranging from 10, 25, 50, 75 and 100µg/kg. General symptoms of toxicity and mortality in each group were observed for 72 h.[15] Animals that survived after 72 h were observed for any signs of Nervousness, Ataxia, Hair Loss, Excitement, Dullness and Death**.** For the further study below 50µg/kg was selected.

Experimental Design

Group 1: Inflammation Group. [Inflammatory agent alone]

Group 2: Vehicle Group [0.2ml/animal i.p., 50% of Ethanol]

Group 3: Standard Drug Group Diclofenac sodium + Inflammation **[**20µg/kg i.p.]

Group 4: Treated Group [40µg/kg i.p., Essential oil of *Tridax procumbens +* Inflammation]

Xylene induced ear inflammation in Swiss Albino Rats

Swiss Albino Rats (250-300g) were divided into four groups (5animals / Group). Animals were treated Intra peritoneally with the essential oil of *Tridax procumbens* 40µg/kg i.p., to group 4, Diclofenac 20µg/kg to group 3 and 0.2ml/animal of 50% ethanol to Group 2 and group 1 serves as a inflammation control. Thirty minutes later, inflammation was induced in each rat 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. The anti-inflammatory activity was expressed as the % inhibition of inflammation in the treated rats in comparison with the control rats.[16]

Carrageenan - Induced paw inflammation in Swiss Albino Rats

Anti-inflammatory activity of *Tridax procumbens* was assessed by Carrageenan induced paw inflammation method. Swiss Albino Rats were divided into 4 groups (5 animals / group). 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) and group II received 0.2ml/animal of 50% ethanol i.p., 30 min before Carrageenan injection. Group III, was given the standard drug Diclofenac 20µg/kg 30 min before Carrageenan injection. Group IV received the essential oil of *Tridax procumbens*40 µg/kg i.p.30 min prior to Carrageenan injection, respectively[17]. The paw volume of the Rats was measured using Vernier caliper prior and after every 3 hour from 1st - 24th hour of Carrageenan injection.

Egg - albumin- induced inflammation in Swiss Albino Rats

Swiss Albino Rats 250-300g of either sex randomized into 4 different groups of 5 rats each were used for the experiment[18]. Animals were treated Intra peritoneally with the essential oil of *Tridax procumbens* 40 µg/kg to group 4, Diclofenac 25µg/kg to group 3 and 0.2ml/kg of 50% ethanol to Group 2 and group 1 served as the Inflammation control. 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 1st to 24th hour after the administration of the phylogistic agent using Vernier caliper.

Histopathological analysis

After euthanasia, the organs were collected in 10% buffered Formalin (Legs and Ears). Then fixed and embedded in paraffin. Tissues were then cut at 5 µm thickness using microtome and the paw and ear skin was excised out, stained with haematoxylin and eosin as per the standard procedure. The slides were examined under light microscope for histopathological changes. The slide examination was performed and reported by the experienced pathologist.

GC-MS analysis

GC-MS analysis was performed as given earlier.[7]

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.

Table 1: Minimum Inhibitory Concentration (MIC) of Essential oil of *Tridax procumbens* on selected Bacterial species

S. No.	Bacterial species	Concentration of essential oil of *Tridax procumbens. L* (µg/ml)				
		25	50	75	100	Negative Control (50% Ethanol)
1	*Escherichia coli*	-	+	+	+	-
2	*Staphylococcus aureus*	+	+	+	-	-
3	*Pseudomonas aeruginosa*	+	+	-	-	-
4	*Streptococcus pneumoniae*	-	+	-	-	-

Table 2: Zone of Inhibition (ZOI) of Essential oil of *Tridax procumbens* on selected Bacterial species

S. No.	Cultures and Dilution used (10^{-6})	Zone of inhibition (mm) of *Tridax procumbens.* L				
		50µg/ml	Positive Control			Negative Control
			Norfloxacin10µg/ml	Cefepime30µg/ml	Gatifloxacin5µg/ml	
1	*Escherichia coli*	16±2.00	26.6±4.16	22.6±1.52	20±4.35	-
2	*Staphylococcus aureus*	16±1.0	32.6±7.5	28±2.00	25.5±0.577	-
3	*Pseudomonas aeruginosa*	16±1.0	29±2.64	13±2.64	12±2.0	-
4	*Streptococcus pneumoniae*	15.3±4.16	28±2.0	21.6±3.51	20.3±0.57	-

Table 3: Minimum Fungicidal Concentration (MFC) of Essential oil of *Tridax procumbens* on selected fungal species

S. No.	Fungal species	Concentration of essential oil (µg/ml)				
		25	50	75	100	Negative Control (50% Ethanol)
1	*Candida albicans*	-	+	+	+	-
2	*Candida parapsilosis*	+	+	+	-	-
3	*Candida tropicalis*	+	+	-	-	-

Table 4: Zone of Inhibition (ZOI) of Essential oil of *Tridax procumbens* on selected fungal species

S. No.	Cultures and Dilution used(10^{-4})	Zone of inhibition (mm) of *Tridax procumbens. L*		
		Essential oil 50µg/ml	Positive ControlFluconozole25 µg/ml	Negative Control(50% Ethanol)
1	*Candida albicans*	15±2.64	17±2.64	-
2	*Candida parapsilosis*	13.6±0.57	18.33±4.163	-
3	*Candida tropicalis*	12±2.0	14.6±0.57	-

Table 5: Acute Toxicity Study of Essential oil of *Tridax procumbens* in Swiss albino Rats

S. No	Concentration of the essential oil	Observations (24 – 72 Hours)					
		Nervousness	Ataxia	Hair Loss	Excitement	Dullness	Death
1	10	-	-	-	-	-	-
2	25	-	-	-	-	-	-
3	50	-	-	-	-	-	-
4	75	-	-	✓	-	✓	-
5	100	-	✓	-	✓	✓	-

Table 6: Effect of Essential oil of *Tridax procumbens* on Carrageenan Induced inflammation in Swiss Albino Rats

Treatment Dose	Readings in time intervals (In cm) (In %)						
	0th Hour	1st Hour	3rd Hour	6th Hour	9th Hour	12th Hour	24thHour
Group I	0.62±0.01	1.17 (58)±0.01	1.13 (53)±0.01	1.12 (52)±0.01	1.05 (52)±0.01	1.04 (49)±0.08	1.01(49)±0.05
Group II	0.62±0.01	1.17 (53)±0.01	1.13 (51)±0.01	1.05 (50)±0.01	0.84 (49)±0.05	0.79 (46)±0.04	0.76(46)±0.05
Group III	0.62±0.01	1.16 (49)±0.02	1.10 (43)±0.04	0.87 (39)±0.05	0.84 (34)±0.38	0.79 (31)±0.04	0.74(27)±0.05
Group IV	0.62±0.01	1.13 (50)±0.03	0.89 (48)±0.08	0.87 (42)±0.06	0.87 (38)±0.05	0.76 (36)±0.05	0.74(30)±0.06

Table 7: Effect of Essential oil of *Tridax procumbens. L* on Xylene Induced inflammation in Swiss Albino Rats

Treatment Dose	Weight of Right ear(g)	Weight of Left ear(g)	Increase in ear weight (g)	% Increase in ear weight	% Inhibition
Group I	0.13±0.009	0.18±0.035	0.05	53	-
Group II	0.12±0.008	0.18±0.035	0.06	50	-
Group III	0.17±0.039	0.22±0.028	0.05	29.4	56.8
Group IV	0.18±0.037	0.22±0.046	0.04	18.1	34

Table 8: Effect of Essential oil of *Tridax procumbens* on Egg- Albumin Induced inflammation in Swiss Albino Rats

Treatment Dose	Readings in time intervals (In cm) (In %)						
	0th Hour	1st Hour	3rd Hour	6th Hour	9th Hour	12th Hour	24th Hour
Group I	0.62±0.01	1.17 (54)±0.01	1.14 (53)±0.01	1.10 (52)±0.05	1.10 (51)±0.05	1.07 (51)±0.007	1.05 (50)±0.01
Group II	0.62±0.01	1.07 (51)±0.007	0.96 (49)±0.003	0.86 (48)±0.002	0.83 (47)±0.02	0.82 (45)±0.01	0.81 (44)±0.01
Group III	0.62±0.01	1.07 (42)±0.08	1.01 (39)±0.04	0.89 (30)±0.04	0.83 (28)±0.01	0.81 (23)±0.004	0.74 (18)±0.01
Group IV	0.62±0.01	1.08 (46)±0.005	0.93 (42)±0.02	0.92 (38)±0.01	0.85 (32)±0.005	0.76 (28)±0.01	0.73 (21)±0.02

Table 9: GC - MS Peak Report of Essential oil of *Tridax procumbens*

Peak S. No.	Retention Time	Initial Time	Final Time	Area	Area%	Height	Height%	Area/ Height	Name of the Compound
1	3.089	3.025	3.125	1404977	2.32	537156	4.90	2.61	-
2	3.195	3.125	3.283	6563421	10.84	916145	8.35	7.16	*Alpha pinene*
3	3.546	3.283	3.575	1265023	20.90	913927	8.33	13.84	*1,3,6- octa triene*
4	3.915	3.850	3.697	741983	1.23	384703	3.51	1.92	*Camphene*
5	4.704	4.575	4.750	2568438	4.24	664050	6.05	3.86	*Beta pinene*
6	5.038	4.867	5.083	4227740	6.98	830028	7.56	5.09	*Sabinene*
7	6.196	5.983	6.225	5838503	9.64	850461	7.75	6.86	*Phellandrene*
8	7.050	6.933	7.092	1520675	2.51	433910	3.95	3.50	*L-limonene*
9	7.272	7.200	7.325	398623	0.66	148684	1.36	2.68	
10	8.173	8.100	8.225	407408	0.67	157430	1.43	2.58	*Beta ocimene*
11	8.406	8.367	8.442	47304	0.08	21588	0.20	2.19	
12	8.666	8.592	8.708	342062	0.57	133368	1.22	2.56	*Trans- beta ocimene*
13	9.125	9.042	9.183	626652	1.04	225624	2.06	2.77	*Phellandrene*
14	9.516	9.472	9.558	86310	0.14	35713	0.33	2.41	
15	15.712	15.667	15.767	72154	0.12	30314	0.28	2.38	
16	17.090	17.042	17.133	70168	0.12	28196	0.26	2.48	
17	18.661	18.617	18.700	57310	0.09	23206	0.21	2.46	
18	18.824	18.717	18.875	982812	1.62	254036	2.32	3.86	*Trans-Caryophyllene*
19	18.932	18.833	18.992	87224	0.14	31481	0.29	2.77	
20	21.022	20.958	21.075	116597	0.19	36966	0.34	3.15	
21	21.311	21.258	21.375	135346	0.22	39441	0.36	3.43	
22	22.372	22.233	22.433	1635788	2.70	326448	2.98	5.01	
23	23.143	23.017	23.192	1185604	1.96	265548	2.42	4.46	*Gama-elemene*
24	23.985	23.908	24.042	290689	0.48	82638	0.75	3.51	
25	28.318	28.175	28.375	2133074	3.52	414454	3.78	5.14	*Torreyol*
26	29.706	29.633	29.775	360907	0.60	95994	0.87	3.75	
27	29.992	29.875	30.042	1379614	2.27	302025	2.75	4.55	*Aromadendrene*
28	31.044	30.983	31.092	97260	0.16	28416	0.26	3.42	
29	31.628	31.558	31.683	196750	0.33	54490	0.50	3.61	
30	33.495	33.400	33.542	1137760	1.88	233704	2.13	4.86	
31	33.606	33.542	33.692	1521607	2.51	328224	2.99	4.63	
32	33.889	33.817	33.958	174034	0.29	42688	0.39	4.07	
33	34.813	34.717	34.875	650562	1.07	154406	1.41	4.21	
34	35.413	35.350	35.475	163379	0.27	45234	0.41	3.61	
35	35.767	35.650	35.833	1221524	2.02	265986	2.42	4.59	*Spathulenol*
36	36.364	36.292	36.433	279495	0.46	77542	0.71	3.60	
37	36.616	36.525	36.683	329581	0.54	73692	0.67	4.47	
38	37.192	37.142	37.350	45179	0.07	18910	0.17	2.38	
39	37.427	37.350	37.583	441123	0.013	61733	0.56	7.14	
40	37.770	37.708	37.833	110605	0.18	28861	0.26	3.83	

RESULTS

The extraction of Essential oil of *Tridax procumbens* from fresh leaves carried out using Clevenger apparatus by Hydro – distillation method gave the yield of 1ml/200gms of the fresh leaves. Table1 shows the Minimal Inhibitory Concentration (MIC) of the essential oil against the bacterial strains which has been taken for the study. Out of the different concentrations ranging from 25, 50, 75 and 100µg/ml analyzed we found that 50µg/ml is the minimal dose to inhibit the growth of microorganisms and the negative control used is 50% of Ethanol.

The anti-microbial activity of the essential oil of *Tridax procumbens* has been evaluated by employing Disc diffusion method in which the activity of the essential oil that is the zones were compared with the three standard antibiotic discs which is susceptible towards the organisms used for the study. Table 2 shows the Zone of Inhibition (ZOI) of the essential oil shows good antibiotic activity (16mm) in comparison with the standard antibiotic discs of Norofloxacin (26.6mm). Interestingly, Cefepime and Gatifloxacin show the less activity for *Pseudomonas aeruginosa* when compared with essential oil of the plant. This shows that the essential oil of *Tridax procumbens* have a significant anti-bacterial activity.

Table 3 shows the Minimal Fungicidal Concentration (MFC) of the essential oil of *Tridax procumbens* against selected fungal species for the study. The concentrations employed for the determination of MFC were 25, 50, 75 and 100 µg/ml in which 50µg/ml was found to be effective against the fungal growth and was used for further analysis of anti-fungal activity.

Table 4 shows the Anti-fungal activity of the essential oil against the selected fungal species when compared with the standard anti-fungal disc Fluconozole. It is found that *Candida albicans* is more susceptible to essential oil with the inhibitory zone of 15mm which is very much equivalent to positive control with 17mm. Next to *C.albicans,* the fungi *C.parapsilosis* and *C.tropicalis* is also found to be susceptible to the essential oil with the zone of 13.6 and 12mm. The

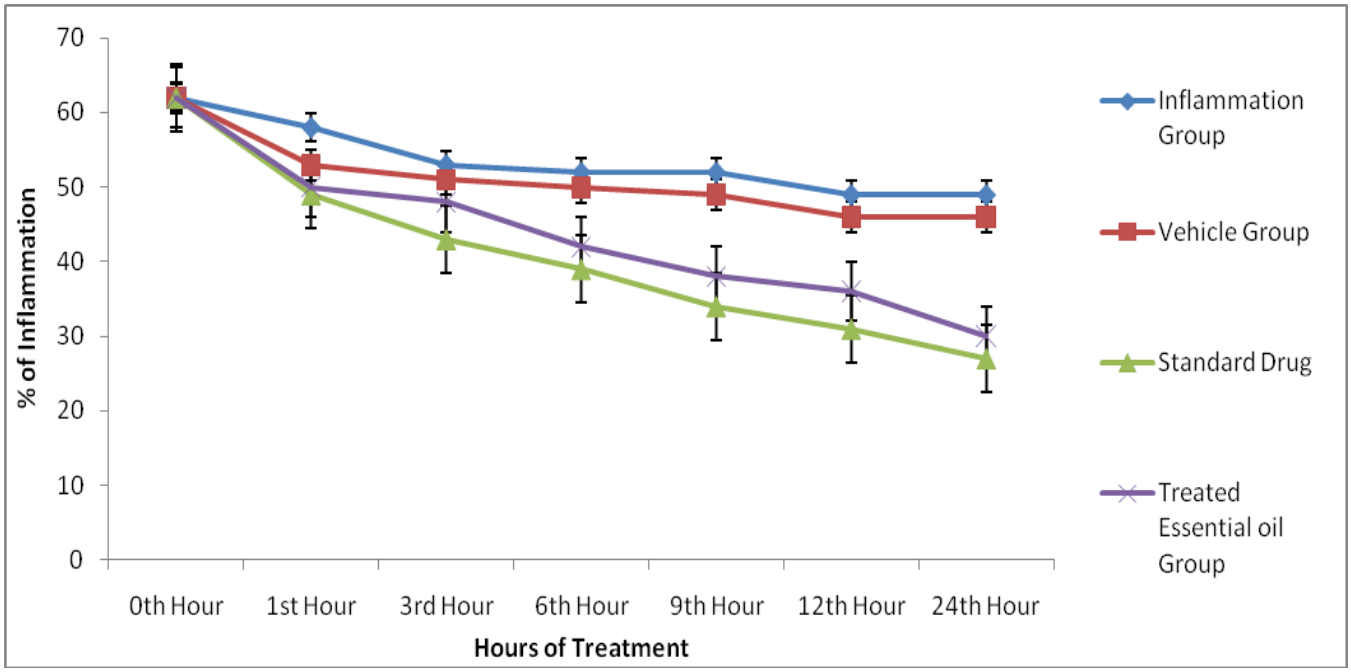
results indicate that the essential oil of *Tridax procumbens* is having a significant antifungal activity too.

Table 5 shows the observations on acute toxicity study of essential oil of *Tridax procumbens* to determine the dosage for evaluating the anti-inflammatory activity of the plant. From the data a dosage of 50µg/ml was found to be stable and no adverse effect has been found in this dosage after 24 – 72 hours of observation and hence this dosage has been finalized for the animal studies.

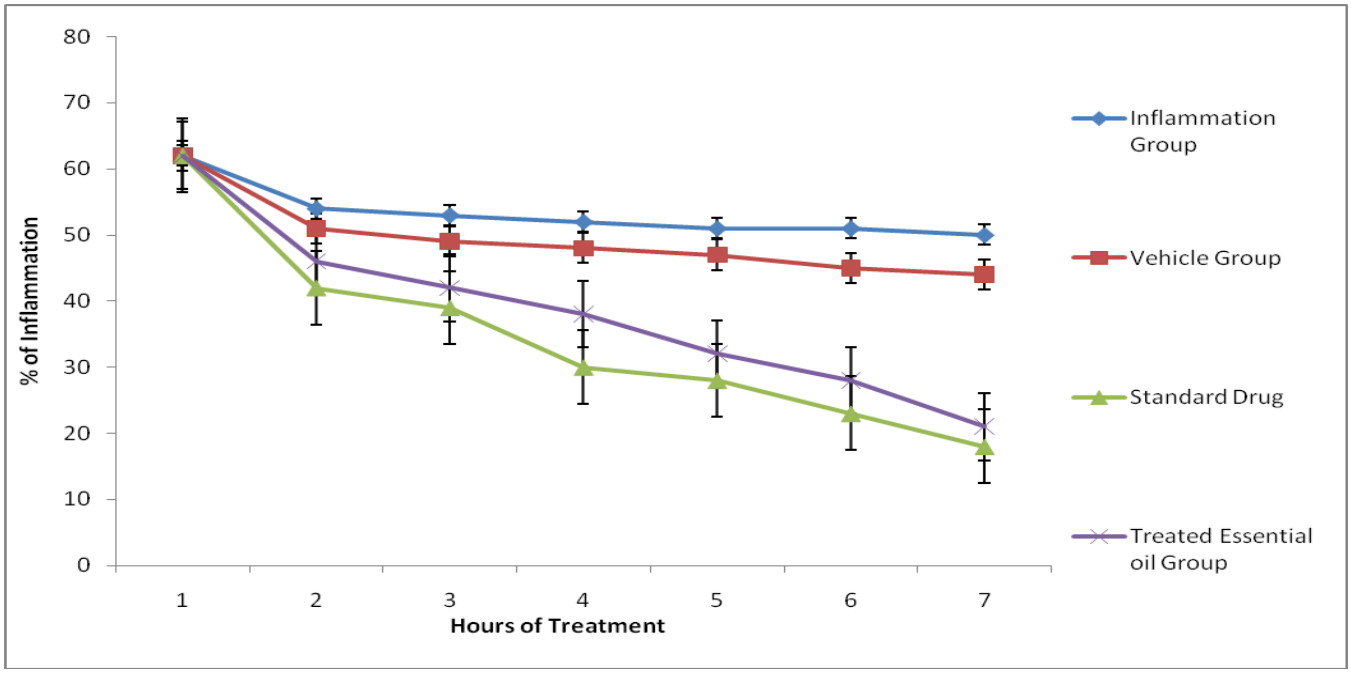
Table 6 and Graph 1 shows the activity of essential oil of *Tridax procumbens* on Carrageenan induced inflammation in rats in which the negative control shows a delayed decrease in the inflammation whereas the essential oil of Tridax shows the gradual and faster decrease in the inflammation with respect to time interval just like standard anti inflammatory drug Diclofenac. Similar result is obtained in egg albumin induced inflammation as shown in Table 8 and Graph 2.

However, the essential oil shows a very good anti inflammatory effect (34%) when compared to Diclofenac (56.8%) in xylene induced inflammation in rat ear in Table 7.

Table 9 and fig 1 shows the result of GC-MS analysis of the essential oil of *Tridax procumbens.* It was found that the oil contains 15 compounds, off which α -pinene, β-pinene, l- phellandrene, Sabinene were the major compounds of 96% equal comparison with the Willey and NBS library.



Graph 1: Effect of Essential oil of *Tridax procumbens. L* on Carrageenan Induced inflammation in Swiss Albino Rats



Graph 2: Effect of Essential oil of *Tridax procumbens. L* on Egg- Albumin Induced inflammation in Swiss Albino Rats

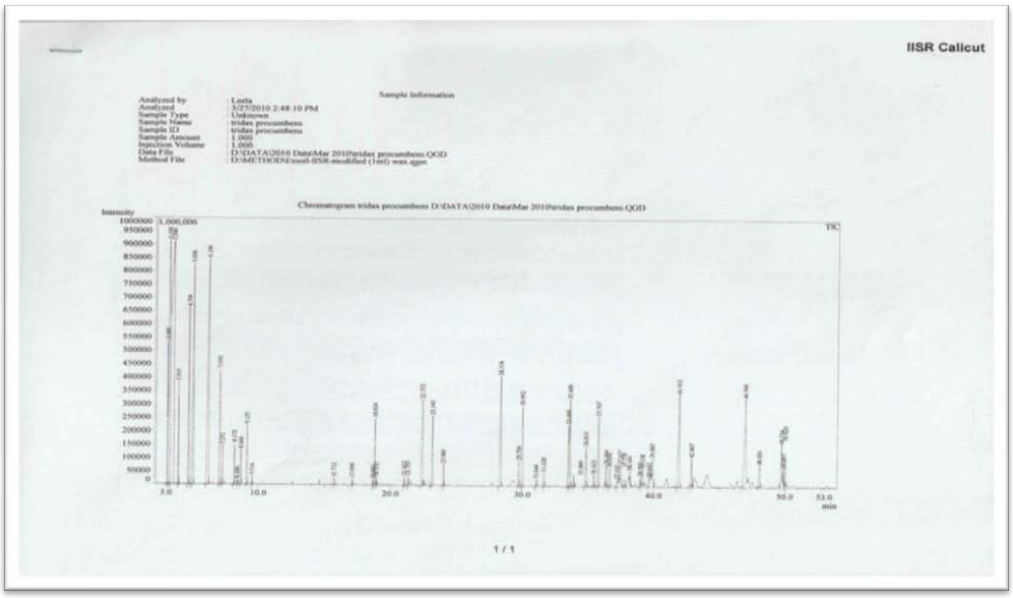


Fig. 1: Results of GC-MS Analysis of Essential Oil of *Tridax Procumbens. L*

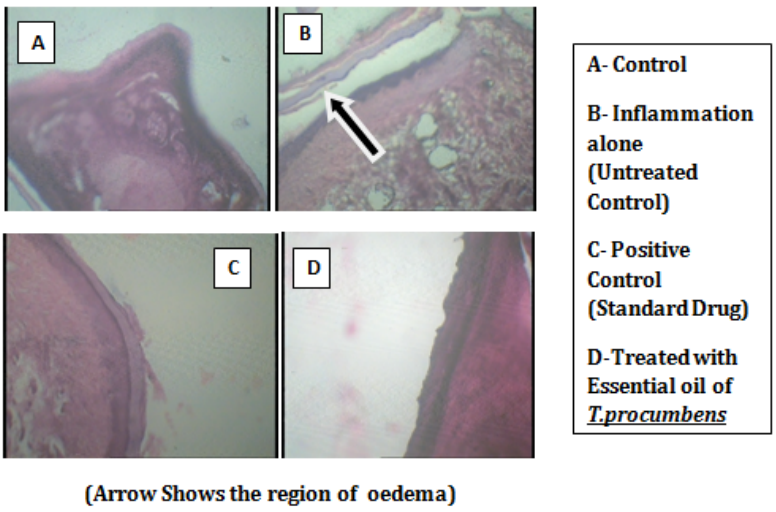


Fig. 2: Histopathological Studies of Inflammation in Legs of Swiss Albino Rats

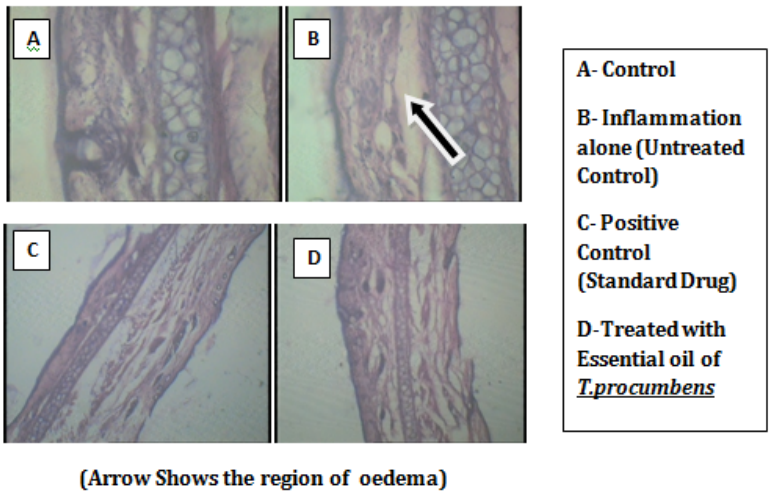


Fig. 3: Histopathological Studies of Inflammation in Ears of Swiss Albino Rats

DISCUSSION

Essential oils or 'essences' are highly concentrated substances extracted from various parts of aromatic plants and trees. Unlike ordinary vegetable oils, such as corn and olive, plant essences are highly volatile and will evaporate if left in the open air. Essential oil is endowed with antiseptic, antibacterial, antibiotic, antidepressant, analgesic, decongestant and sedative properties. Moreover, due their tiny molecular structure, essential oils applied to the skin can be absorbed into the bloodstream. They also reach the blood as a result of the aromatic molecules being inhaled. In the lungs, they pass through the tiny air sacs to the surrounding blood capillaries by the process of diffusion.

The essential oils produced by the plants have been used traditionally for the respiratory tract infections and in the recent centuries the oil is used as ethical medicines for cold and microbial infections. By inhaling the vapors of the essential oil it shows the greater output of respiratory fluid and hence it is used in treating acute and chronic bronchitis, acute sinusitis and has an anti-inflammatory effect on trachea and reduced asthma[19].

Earlier researchers reports that α - and β-pinenes are active against yeast and bacteria and this mechanism lies mainly in their capacity to induce toxic effects on the membrane structure and functions. Moreover, α-pinenes are used against mushrooms and yeasts (dermatophytes) 20, especially on *Candida albicans*21 and other related species such as *Candida tropicalis*, *C. glabrata*22 etc. The β-pinenes also show antifungal properties 23, especially on *Candida* spp.24

Studies have confirmed that the essential oil which is rich in alpha – pinene shows a potential anti - bacterial and anti - fungal activity[25]. The major components of our plant *Tridax procumbens* is also alpha – pinene which showed significant anti – bacterial and anti – fungal activity against the bacterial and fungal strains taken for the study.[29] Moreover, another study confirmed that monoterpenes and terpinenes have also shows antimicrobial properties that appear to have a strong anti – microbial activity against gram positive bacteria [26]. Researchers have confirmed that the bridged activity of this bi-cyclic monoterpenes alpha and beta pinenes possesses a considerable anti – microbial activity[27]. The anti - microbial activity showed by the essential oil of Tridax procumbens may be due to the presence of this monoterpenes as its major compounds and hence the result confirms from the earlier studies that the presence of alpha and beta pinene[30] is the confirmatory evidence that the study plant material essential oil has a significant anti – microbial activity.

Carrageenan, Egg-albumin and Xylene induce inflammation in animal models like mice and rats are used for the assessment of anti-inflammatory activity of any formulated drug[28]. When α- and β-pinenes are the major constituents of an essential oil, they warrant the anti-inflammatory and analgesic activity too[29].

In this study, our study was on the role and activity of essential oil against different inflammatory agents used for the study. In our study, we found that the *Tridax procumbens* also contains the same compound as its major compound and the activity shown by the essential oil may also be due to the presence of these terpenes as its major compounds.

CONCLUSION

Tridax procumbens is a very commonly used medicinal plant for wound healing and other inflammations even in eyes in India. The essential oil of *Tridax procumbens* has two major volatile compounds such as Alpha – pinene and beta - pinene, which pose no hazard when it is used in small quantities. The essential oil of *Tridax procumbens* has a number of properties that are beneficial to human health and wellbeing. It may be used in the pharmaceutical and cosmetic industries for the development of new drug formulation for treating bacterial and fungal infections and also for treating inflammation related diseases in a cheaper rate with fewer side effects.

ACKNOWLEDGEMENT

Authors acknowledge the valuable help rendered from Dr. G.V.S. Murthy (Joint Director) Botanical Survey of India –Coimbatore-T.N.A.U Campus, Tamilnadu for Identification and Authentication of the plant specimens and DR.N.K. Leela (Senior Scientist) – GC-MS Survey, *Indian Institute of Spices Research* (IISR) , Calicut Kerala for GC-MS Analysis and Validation of the results and Dr. Prabhu, (Dr. N. G. P. Arts and Science College), Coimbatore, Tamilnadu for providing fungal cultures. Authors acknowledge the valuable suggestions and technical guidance given by Dr. C. Guruvayoorappan, Assistant Professor (SG), Department of Biotechnology, Karunya University, Coimbatore. I am grateful to Mr.Siddikuzzaman and M/s. D. Ramya for their technical support during the research.

REFERENCES

1. Nia. R., Essiel. E.E., Investigation into in vitro radical scavenging and *in vivo* anti- inflammatory potential of Tridax procumbens. *Nigerian Journal of physiological sciences*. 2000; 18(1):39-43.
2. Habila. J.D., Bello. I.A., Dzikwi. A.A., Total phenolics and anti oxidant activity of *Tridax procumbens. Linn*. *African Journal of Pharmacy and Pharmacology*. 2010; 4(3):123-126.
3. Sneha mundada., Ruchi shivahare., Pharmacology of *Tridax procumbens* a weed : Review. *International Journal of Pharm Tech Research.* 2010; 2(2):1391- 1394.
4. Sharma. B., Kumar. P., Extraction and pharmacological evaluation of some extracts of Tridax procumbens and capparris deciduas. 2009; 1(4): 5-12
5. Ikewuchi Jude., Ikewuchi Catherine. C., International Journal of applied Research in Natural products, chemical profile of *Tridax procumbens Linn. Pakistan Journal of Nutrition.* 2009; 8(5): 548-550.
6. Manjamalai. A, V.M. Berlin Grace, Analysis of Phytochemical Constituents and Anti-Microbial Activity of Some Medicinal Plants in Tamil Nadu, India. *Global Journal of Biotechnology & Biochemistry.* 2010; 5(2): 120-128.
7. Shatish Kumar.k., Extracting of essential oil using steam distillation. Institute of Technology (B-Tech project). 2010:P: 1-33
8. Sarfaraz Khan Marwat, Muhammad Aslam Khan., Aromatic plant species mentioned in the holy Quran and Ahadith and their Ethno medicinal importance, *Pakistan journal of Nutrition.* 2009; 8(9): 1472-1479.
9. Pandy. D., Raovirendra. P.S., Extraction of Essential oil from Eucalyptus leaves; (B-tech project); NIT- Rourkela.
10. Amr E., Edris-Pharmaceutical and Therapeutic Potential of Essential oils and their individual Volatile constituents; A Review Phytotherapy Research, 2007.
11. Extraction Technologies for Medicinal and Aromatic plants United Nations Industrial Development Organization and the International Centre for Science and High Technology, 2008.
12. Khadidja Mazari, Nassima Bendimerad, Chahrazed Bekhechi and Xavier Fernandez, Chemical composition and antimicrobial activity of essential oils isolated from *Algerian Juniperus phoenicea L and Curessus sempervirens Journal of Medicinal Plants Research.* 2010; 4(10): 959-964.
13. Fernanda Braga G., Maria Lucia Bouzada. M, Anti - Leishmanial and antifungal activity of plants used in traditional medicine in Brazil; *Journal of Ethno pharmacology.* 2007; 111: 396-402.
14. Guidance for Industry. Single Dose Acute Toxicity Testing for Pharmaceuticals-Centre for Drug Evaluation and Research (CDER). 1996; P. 1-2.
15. Ighodaro Igbe, Fidelis P. Ching and Aigbe Eromon. Anti-inflammatory activity of aqueous fruit pulp extract of *Hunteria umbellata* in acute and chronic inflammation. *Acta Poloniae Pharmaceutica* - Drug Research, 2010; 67(1) :81-85.
16. Winter C.A, Riley EA., Anti-inflammatory and antipyretic activities of indomethacin; *Journal Pharmacology and Experimental Therapy*, 1963; 141-269.
17. Jude E Okokon and Paul A., Anti-inflammatory, Analgesic and Antipyretic Activities of Ethanolic Root Extract of *Croton Zambesicus Pakistan Journal of Pharmaceutical Science*: 2010; 23(4): 385-392.
18. Sandeep Biradar, V.A. Kangralkar**.** Anti-inflammatory, anti arthritic, analgesic and anticonvulsant activity of Cyprus essential oil *International journal of pharmacy and pharmaceutical science*; 2010; 2(4)

19. Mahmet Karaca., Hanefi osbek., Investigation of anti-inflammatory activity of bergamot oil *European journal of general medicine*; 2007; 4(4); 176-179.
20. Maria Graça Miguel. Antioxidant and Anti-Inflammatory Activities of Essential Oils:
21. A Short Review; *Molecules*; 2010; 15; 9252-9287
22. Ibraliu,X.Mi. Analysis of essential oils of three wild medicinal plants in Albania *Journal of medicinal plants research*; 2011; 5(1); 58-62
23. Zahid zaheer,Khan Subur. Microbial activity of essential oil of flowers of *Pluemmera Alba Linn, International journal of pharmacy and pharmaceutical science*; 2010; 4(2)
24. M.Vasinauskiene, J.Radusiene. Antibacterial activity of essential oils from aromatic and medicinal plants against growth of phytopathogenic bacteria; *Agronomy research*; 2006; 4; 437-440
25. Afolan. A. J, Ashafa.A. Chemical composition and antimicrobial activity of the essential oil from *Chrysocoma ciliate* L. leaves; *Journal of medicinal plants research*; 2009; 3(5); 390-394
26. Beatrice Mercier, Julianne prost. The essential oil of turpentine and its major volatile fraction (alpha and beta pinene): a review; *International journal of occupational medicine and environmental health*; 2009; 22(4), 231-242
27. Sudha parimala., Shashidhar. G., Anti - inflammatory activity of *Celastrus paniculatus* seeds, *International journal of Pharma tech research*; 2009; 4(1), 1326-1329
28. Sandeep Biradar., Kangralkar., V. A., Anti-inflammatory, Anti-arthritic, Analgesic and anticonvulsant activity of Cyprus essential oils; *International journal of pharmacy and pharmaceutical science*, 2010; 4(2):132-138.
29. Chiron F, Chalchat JC, Garry RP, Pilichowski JF, Lacoste J. Phytochemical hydro peroxidation of terpenes. I. Synthesis and characterization of α-pinene, α-pinene and limonene hydro peroxides, *Journal of Photochemical Photo biology A: Chemistry* .1997; 111(1–3): 75–86.
30. Sukantha T.A, Shubashini K. Sripathi Antioxidant and Antibacterial Activities of Trianthema Decandra Linn, International Journal of Pharmacy and Pharmaceutical Sciences, 2012; 4(1): 410-413.
31. Raghuveer Rodda., Abeesh Kota Investigation on Anti Inflammatory Property of Basella Alba Linn Leaf Extract, International Journal of Pharmacy and Pharmaceutical Sciences, 2012; 4(1): 452-454.