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Research Article

STUDY ON THE EFFECT OF ESSENTIAL OIL OF *WEDELIA CHINENSIS (OSBECK)* AGAINST MICROBES AND INFLAMMATION

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ABSTRACT

The aim of the study is to evaluate the Anti-Microbial and Anti-Inflammatory activity of the essential oil of *Wedelia chinensis.* Based on the preliminary study, on the determination of Minimal Inhibitory Concentration and Minimal Fungicidal Concentration carried out, 50µg/ml was taken as the optimum dose for the determination of anti-microbial activities of the essential oil. The study was carried out against selected bacterial and fungal strains. The results obtained for the study was compared with the respective standard antibiotic discs of both bacteria and fungi. Inflammation has been induced in the Swiss albino rat models of both sexes using different inflammatory agents in the paw and ear of essential oil pretreated rats. Then the inflammatory site is measured in different time intervals. Before the commencement of the study, acute toxicity study was carried out for identifying the minimal dosage of drug which is not toxic to the animal when administered intraperitoneally. The results obtained for the anti-inflammatory activity was compared with the positive control Diclofenac sodium and negative control (50% of Ethanol). GC-MS analysis was carried out in the essential oil for the identification of the major compounds present in *Wedelia chinensis.* The identification was based on comparison of their mass spectra and retention indices. The major compounds identified were Carvocrol and t- Caryophyllene.

Keywords: Anti-bacterial, Anti-fungal, GC-MS, Carvocrol, Anti-inflammatory, Swiss albino rats

INTRODUCTION

Medicinal plants constitute an undocumented and over exploited economic resource and are the principal health care resource for majority of the people as the oral traditions practiced by the rural villagers. Today we find a renewed interest in traditional medicine to validate scientifically. During the past decade there has been an ever increasing demand especially from developed countries for more and more drugs from plant sources. This renewed interest in plant derived drug is mainly due to the current widespread belief that green medicine is safe and more dependable than the costly synthetic drug with adverse effect. The rural people constitute 70-75% of the Indian population live in about 576000 villages located in different agro-climatic conditions. The village people have their own diverse system of health management. While most of the common ailments were managed in the house itself by home remedies which include many species and condiments like Pepper, Ginger, Turmeric, Coriander, Cummins, Fenugreek, Tulsi etc.[1]

Wedelia chinensis is a traditionally used medicinal herb in Ayurveda, Siddha and Unani system of medicines. The plant leaves contains two major bioactive compounds such as Wedelolactone and dimethyl Wedelolactone which are responsible for the potent anti-hepatotoxic and hence it is used as a major ingredient in phyto-pharmaceuticals formulations. Different solvent extraction of this plant leaves are used for treating osteoporosis of knee and also possess anti-inflammatory activity.[2, 3] As it contains large amount of phenolic constituents, its richness in wound healing activity has been studied by many group of researchers by using aqueous extract of the leaves of this plant on open wound and sutured wound models.[4]

Wedelia chinensis leaves extract are the natural alternative to commonly used anti- inflammatory drug like Dolonex, Brufen etc. and this plant extract can be used with confidence for treating rheumatic fever.[5] The major compound present in the plant leaves have been reported already for its synergistic suppression activity in prostate cancer cells. Extracts from this plant have also been used in treating number of other diseases like elephantiasis, toothache and headache as well as emitics and purgative materials and as external antiseptic. Shoot extract of this plant possess anti-biotic activity against certain species.[6]

Our research group has already identified and reported the major bioactive compounds present in the essential oil of *Wedelia chinensis* using GC and GC-MS analysis[7]. The present study is focused on evaluation of essential oil of *Wedelia chinensis* for its activity against bacterial and fungal strains. We also intended to evaluate the efficiency of essential oil of *Wedelia chinensis* in reducing the inflammation in experimental animal models.

MATERIALS AND METHODS

Plant Material Collection and Authentication

Fresh leaves of the selected plant *Wedelia chinensis* (Osbeck) having medicinal value were 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 *Wedelia chinensis*

Extraction of essential oil from *Wedelia chinensis* is done by Hydro distillation method 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 gets separated based on difference in density and immiscibility, which is then collected and dried over anhydrous sodium sulphate and stored in vial at low temperature until analysis.[8]

ANTI-MICROBIAL ACTIVITY

Preparation of Inoculum

The bacterial and fungal cultures were obtained from Dr. N. G. P. Arts and Science College, Coimbatore, Tamil Nadu 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 and fungal cultures were maintained in Potato dextrose agar plates and slants. All the cultures were further sub cultured before use. The mother inoculum was maintained at 37^0C for 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) on bacteria

The Minimal Inhibition Concentration (MIC) values were determined against the bacterial strains for the essential oil of *Wedelia chinensis.* A 100µl of the inoculum, initially adjusted to 10^{-6} CFU/ml, was spread onto 20 ml 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.[9]

Determination of Minimum Fungicidal Concentration (MFC)

The Minimal Fungicidal Concentration (MFC) values were determined against the fungal strains for the essential oil of *Wedelia chinensis.* The MFC was determined by incorporating various concentration of essential oil 25, 50, 75 and 100 µg/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.[10]

ANTIBACTERIAL SCREENING

Disc diffusion method

The disc diffusion method was employed for the determination of antibacterial activities of the essential oil. Paper discs (6 mm diameter) were impregnated with 50 µg/ml of the oil dissolved in 50% Ethanol to 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.[9]

ANTIFUNGAL SCREENING

Disc diffusion method

The disc diffusion method was employed for the determination of antifungal activities of the essential oil. Paper discs (6 mm diameter) were impregnated with 50 µg/ml of the oil dissolved in 50% of Ethanol 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 that 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.[9]

ANTI-INFLAMMATORY ACTIVITY

Experimental animals

Swiss Albino Rats (250-300g) were used for the study (5animals/group/cage) and 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 boiled water. All the experiments involving animals were performed according to the standard protocols from National Institute of Nutrition (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. Animals that survived after 72 h were observed for any signs of Nervousness, Ataxia, Hair Loss, Excitement, Dullness and Death.[11]

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 *Wedelia chinensis +* Inflammation]

Xylene induced ear inflammation in Swiss Albino Rats

Swiss Albino Rats (250-300g) were divided into 4 groups (5animals / Group). Animals were treated Intra peritoneally with the essential oil of *Wedelia chinensis* 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 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.[12]

Carrageenan - Induced paw inflammation in Swiss Albino Rats

Anti-inflammatory activity of *Wedelia chinensis* 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 *Wedelia chinensis* 40 µg/kg i.p.30 min prior to Carrageenan injection, respectively [13]. 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. Animals were treated Intra peritoneally with the essential oil of *Wedelia chinensis* 40 µg/kg to group 4, Diclofenac 25µg/kg to group 3 and 0.2ml/animal of 50% ethanol to Group 2 and group 1 served as the Inflammation control[14]. 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.

GC-MS analysis

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

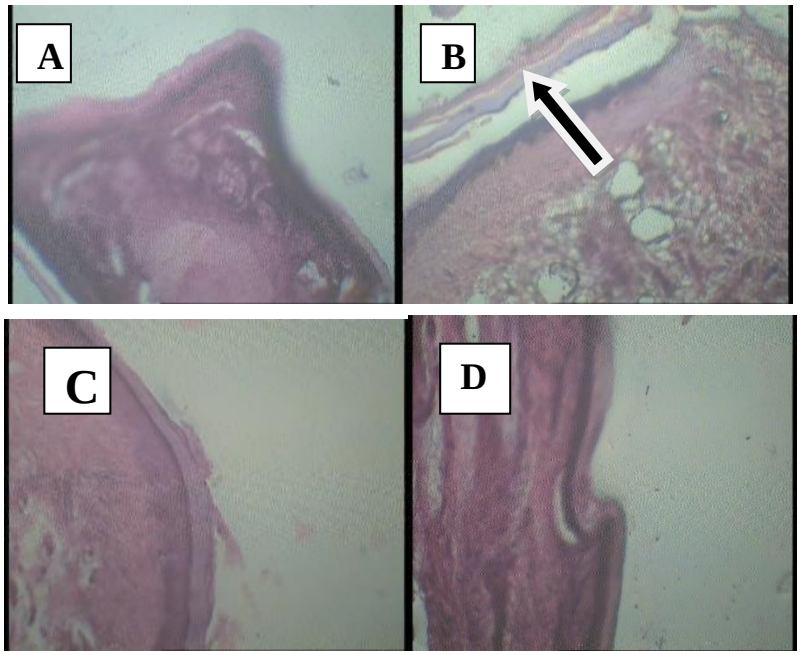
Histopathological analysis

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.

Statistical analysis

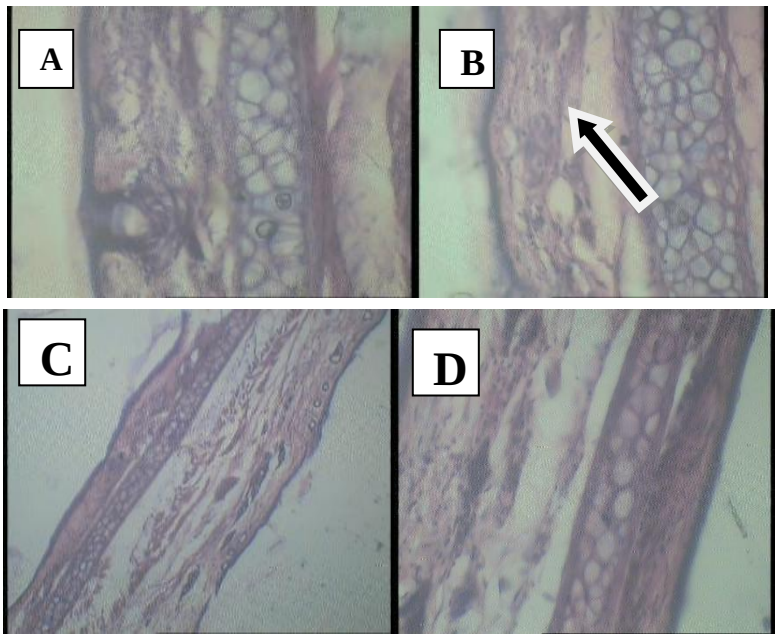
Data was statistically analyzed using one – way ANOVA as primary test followed by Dunnett's test using Graph pad InStat3.0 software for Windows XP, Graph pad Software, San Diego, California, USA.

Figure 1: Histopathological Studies of Inflammation in Legs of Swiss Albino Rats



A- Control
B- Inflammation alone (Untreated Control)
C- Positive Control (Standard Drug)
D-Treated with Essential oil of *W.chinensis*

Figure 2: Histopathological Studies of Inflammation in Ears of Swiss Albino Rats



A-Control
B- Inflammation alone (Untreated Control)
C- Positive Control (Standard Drug)
D-Treated with Essential oil of *W.chinensis*

RESULTS

Essential oil extraction from *Wedelia chinensis* has been done using the fresh leaves of the plant and the extraction was done by using Clevenger trap employing hydro distillation method using water as the solvent at its boiling temperature. The preliminary study includes the antimicrobial activity of the essential oil of the selected organisms for this the determination of MIC and MFC has been carried out for the dose fixation.

Table 1 shows the Minimal Inhibitory Concentration (MIC) of the essential oil of *Wedelia chinensis* against different bacterial species. From the observation 50µg/ml was selected as the minimal dose which has the efficient activity against the bacterial cultures. Table 2 and Graph 1 shows the Zone of Inhibition of the essential oil of *Wedelia chinensis*, with a significant inhibitory activity against *Staphylococcus aureus* with a maximum zone of 16.3mm which is a more or less equal activity to the standard drug. This shows that the essential oil is effective against the bacteria. The other strains used for the study also were susceptible to the essential oil.

Table1: Minimum Inhibitory Concentration (MIC) of Essential oil of *Wedelia chinensis. Osbeck* on selected Bacterial species

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

Table2: Zone of Inhibition (ZOI) of Essential oil of *Wedelia chinensis. Osbeck* on selected Bacterial species

S.NO	Cultures and Dilution used(10^{-6})	Zone of inhibition (mm) of *Wedelia chinensis. Osbeck*				
		50 µg/ml	Positive Control			Negative Control
			Norfloxacin 10µg	Cefepime 30µg	Gatifloxacin 5µg	
1	*Escherichia coli*	14 ±2.0	30.6 ±1,15	21.6 ±1.52	24.3 ±1.52	-
2	*Staphylococcus aureus*	16.3 ±4.04	21.3 ±1.528	14.6 ±0.577	16 ±2.64	-
3	*Pseudomonas aeruginosa*	15.3 ±2.08	29.33 ±6.04	26.6 ±3.51	22.6 ±1.52	-
4	*Streptococcus pneumoniae*	16 ±2.0	29.6 ±0.57	15.3 ±0.577	15 ±0.00	-

Graph: 1 Zone of Inhibition (ZOI) of Essential oil of *Wedelia chinensis. Osbeck* on selected Bacterial species

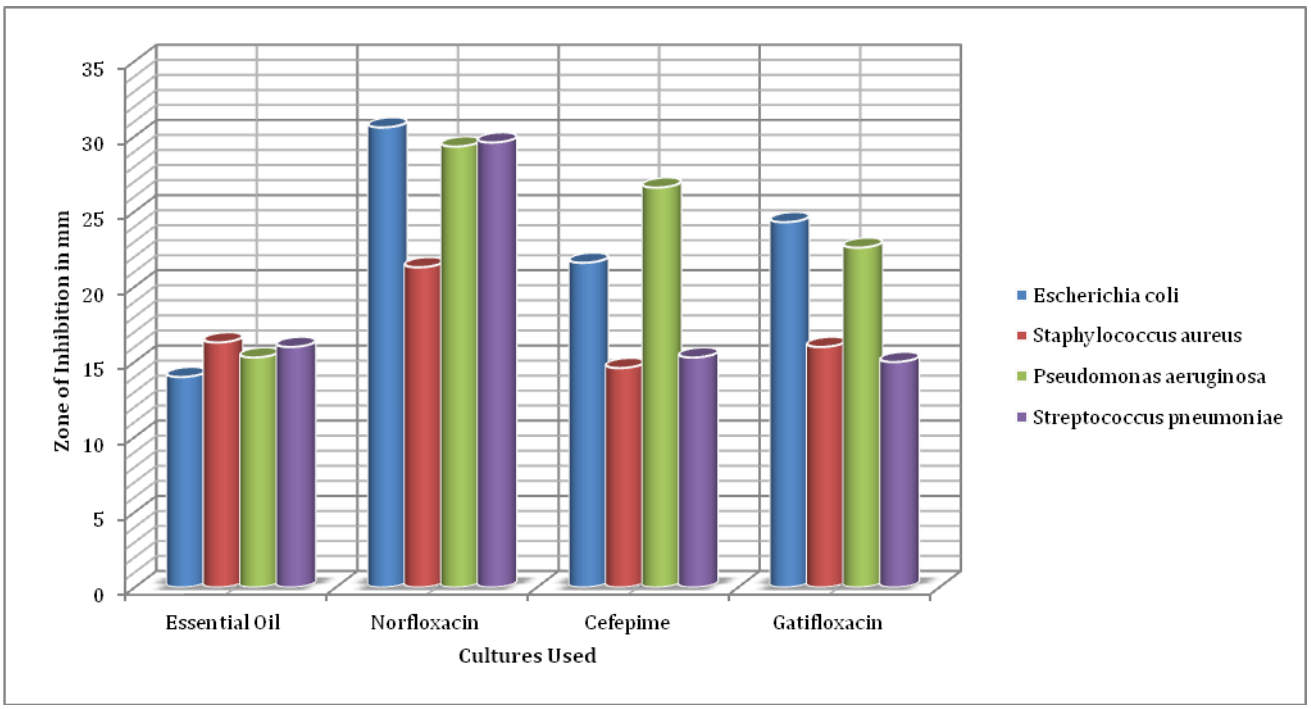


Table3: Minimum Fungicidal Concentration (MFC) of Essential oil of *Wedelia chinensis. Osbeck* on selected fungal species

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 *Wedelia chinensis* on selected fungal species

S.NO	Cultures	Dilution used (10^{-4})	Zone of inhibition (mm) of *Wedelia chinensis. Osbeck* 50 µg/ml	Positive Control Fluconozole 25 µg	Negative Control (50% Ethanol)
1	*Candida albicans*		16 ±1.00	21 ±1.00	-
2	*Candida parapsilosis*		13.3 ±1.15	19.3 ±2.30	-
3	*Candida tropicalis*		18.3 ±5.50	21 ±4.00	-

Graph: 2 Zone of Inhibition (ZOI) of Essential oil of *Wedelia chinensis. Osbeck* on selected fungal species

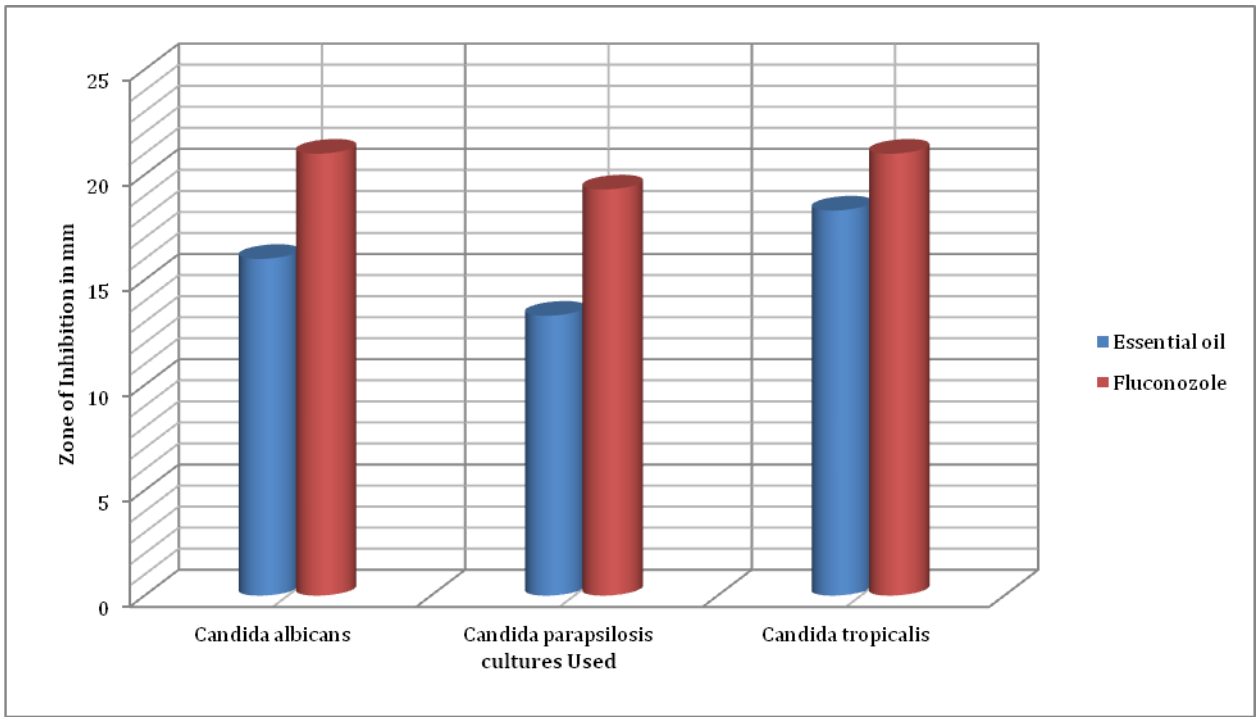


Table 3 infers the Minimal Fungicidal Concentration of the essential oil against the fungal cultures used for the study. The dose of 50µg/ml was found to be the minimal dose that inhibits the growth of the fungal inoculums used for the study. Table 4 and Graph 2 show the Zone of inhibition activity of essential oil against the fungal strains used for the study. The essential oil showed a significant inhibitory activity against the three selected fungal strains when compared with the standard antifungal disc Fluconozole, more or less equal activity.

The minimal dose of 40µg/ml has been taken as the dose for the inflammatory studies based on the acute toxicity study. The study reveals that the drug has a significant anti-inflammatory activity against the inflammatory agent used when compared with the control as shown in Table 5 and Graph 3. Similarly Table 6 and Graph 4 show the significant effect of *Wedelia chinensis* essential oil on Egg-albumin induced inflammation in rats.

Table5: Effect of Essential oil of *Wedelia chinensis. Osbeck* on Carrageenan Induced inflammation in Swiss Albino Rats

TREATMENT DOSE	TIME INTERVALS (IN HOURS) READINGS (In cm) (In %) 0th Hour	1st Hour	3rd Hour	6th Hour	9th Hour	12th Hour	24th Hour
Group I	0.62 ±0.01	1.16 **(49)** ±0.10	1.14 **(46)** ±0.01	1.10 **(44)** ±0.05	1.03 **(40)** ±0.01	1.03 **(40)** ±0.01	1.01 **(38)** ±0.08
Group II	0.62 ±0.01	1.12 **(42)** ±0.10	0.97 **(36)** ±0.10	0.93 **(33)** ±0.1	0.92 **(31)** ±0.02	0.90 **(30)** ±0.02	0.89 **(29)** ±0.06
Group III	0.62 ±0.01	1.14 **(46)** ±0.01	1.02 **(38)** ±0.08	0.90 **(31)** ±0.05	0.84 **(29)** ±0.07	0.81 **(24)** ±0.06	0.79 **(19)** ±0.06

Group IV	0.62 ±0.01	1.13 (47) ±0.10	0.97 **(36)** ±0.10	0.83 **(25)** ±0.1	0.82 **(24)** ±0.02	0.79 (**24**) ±0.02	0.77 **(23)** ±0.06

Graph 3: Effect of Essential oil of *Wedelia chinensis. Osbeck* on Carrageenan Induced inflammation in Swiss Albino Rats

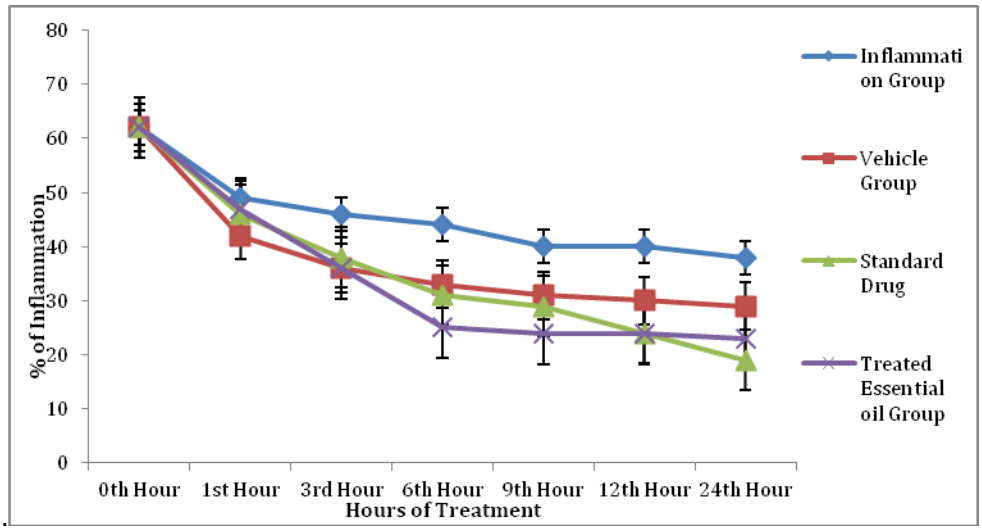
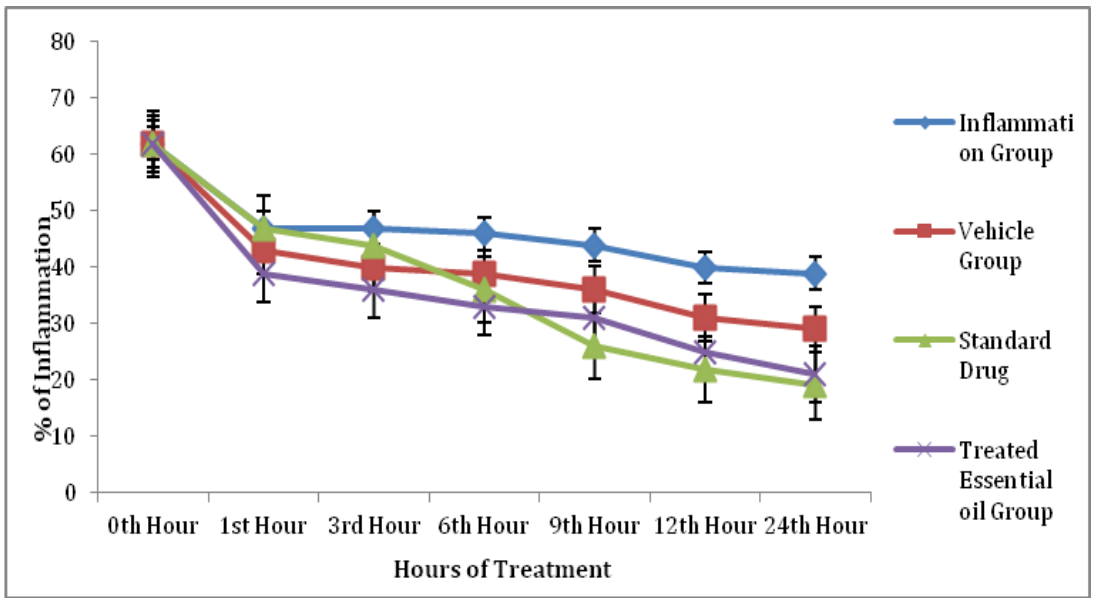


Table6: Effect of Essential oil of *Wedelia chinensis. Osbeck* on Egg- Albumin Induced inflammation in Swiss Albino Rats

TREATMENT DOSE	TIME INTERVALS (IN HOURS) READINGS (In cm) (In %)						
	0th Hour	**1st Hour**	**3rd Hour**	**6th Hour**	**9th Hour**	**12th Hour**	**24th Hour**
Group I	0.62 ±0.01	1.16 **(47)** ±0.06	1.16 **(47)** ±0.06	1.14 **(46)** ±0.01	1.10 **(44)** ±0.05	1.03 **(40)** ±0.01	1.02 **(39)** ±0.09
Group II	0.62 ±0.01	1.08 **(43)** ±0.008	1.03 **(40)** ±0.01	0.97 **(39)** ±0.004	0.95 **(36)** ±0.007	0.83 **(31)** ±0.02	0.82 **(29)** ±0.01
Group III	0.62 ±0.01	1.16 **(47)** ±0.06	1.10 **(44)** ±0.05	0.97 **(36)** ±0.04	0.84 **(26)** ±0.08	0.79 **(22)** ±0.04	0.73 **(19)** ±0.02
Group IV	0.62 ±0.01	1.02 **(39)** ±0.09	0.97 **(36)** ±0.133	0.92 **(33)** ±0.14	0.90 **(31)** ±0.10	0.83 **(25)** ±0.11	0.80 **(21)** ±0.07

Graph: 4 Effect of Essential oil of *Wedelia chinensis. Osbeck* on Egg- Albumin Induced inflammation in Swiss Albino Rats



The Table 7 shows a significant decrease in the weight of the ear in the induced rats with inflammation, which has been treated with the plant essential oil. This shows that the essential oil of *Wedelia chinensis* possesses a significant anti-inflammatory activity.

Table7: Effect of Essential oil of *Wedelia chinensis. Osbeck* on Xylene Induced inflammation in Swiss Albino Rats

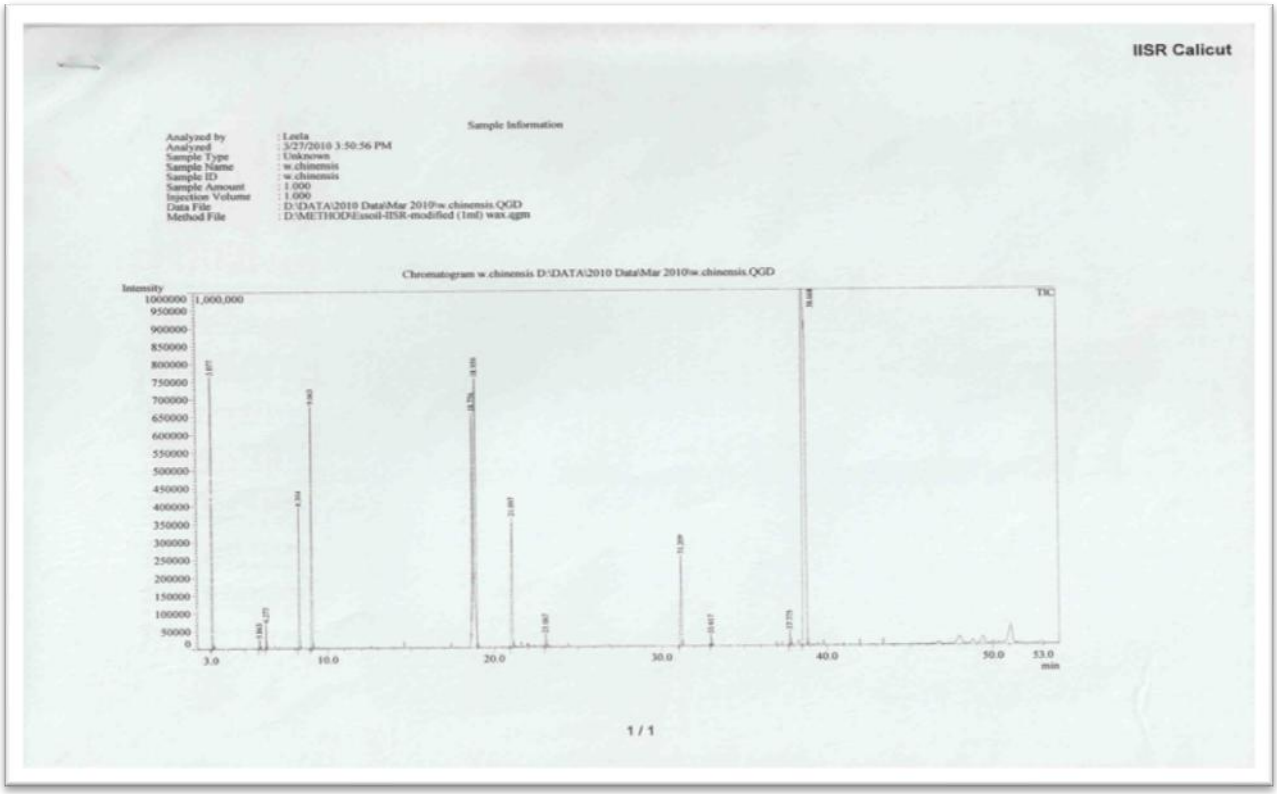
TREATMENT DOSE	Weight of Right ear (g)	Weight of Left ear (g)	Increase in ear weight (g)	% Increase in ear weight	% Inhibition
Group I	0.19 ±0.041	0.18	0.06	53	-
Group I	0.14 ±0.039	0.18 ±0.035	0.04	50	-
Group II	0.17 ±0.039	0.23 ±0.029	0.05	29.4	58
Group III	0.14 ±0.021	0.21 ±0.0395	0.07	33.3	65

Table 9 show the bioactive compounds identified through GC-MS analysis of essential oil of *Wedelia chinensis* fresh leaves. The graph shows that the plant contains nearly 10 compounds, out of which Carvocrol and T – Caryophyllene were found to be the major compound.

Table 8: GC - MS Peak Report of Essential oil of *Wedelia chinensis. Osbeck*

Peak S.No	Retention Time	Initial Time	Final Time	Area	Area%	Height	Height%	Area/ Height	Name of the Compound
1	**3.077**	**3.008**	**3.142**	**2262359**	**7.29**	**766572**	**15.49**	**2.95**	
2	**5.863**	**5.808**	**5.917**	**74913**	**0.24**	**27661**	**0.56**	**2.70**	***β-myrcene***
3	**6.273**	**6.208**	**6.317**	**193397**	**0.62**	**71627**	**1.45**	**2.70**	***α-terpinene***
4	**8.304**	**8.192**	**8.358**	**1416392**	**4.57**	**396503**	**8.01**	**3.57**	***γ- terpinene***
5	**9.063**	**8.942**	**9.108**	**2709933**	**8.73**	**682275**	**13.78**	**3.97**	***Ortho-lymene***
6	**18.756**	**18.633**	**18.783**	**1837973**	**5.92**	**487288**	**9.84**	**3.77**	***α-bergamotene***
7	**18.959**	**18.783**	**19.025**	**4600145**	**14.83**	**703940**	**14.22**	**6.53**	***Trans Caryophyllene***
8	**21.097**	**20.950**	**21.158**	**1765457**	**5.69**	**364894**	**7.37**	**4.83**	***α-humulene***
9	**23.067**	**23.008**	**23.133**	**124813**	**0.40**	**38341**	**0.77**	**3.25**	
10	**31.209**	**31.067**	**31.275**	**1421838**	**4.58**	**257366**	**5.20**	**5.52**	***Aromadendrene***
11	**33.017**	**32.950**	**33.092**	**147489**	**0.48**	**31423**	**0.63**	**4.69**	***3-decynex***
12	**37.775**	**37.708**	**37.850**	**178970**	**0.58**	**43393**	**0.88**	**4.12**	***Thymol***
13	**38.668**	**38.492**	**38.867**	**14293870**	**46.07**	**1078962**	**21.80**	**13.24**	***Carvocrol***

Graph: 5 Results of GC-MS Analysis of Essential Oil of *Wedelia chinensis. Osbeck*



DISCUSSION

The present study carried out using essential of *Wedelia chinensis* against microbes and inflammatory agents showed a significant result due to the major compounds present in the essential oil such as Carvocrol and Trans – Caryophyllene. Carvocrol inhibits the growth of several bacterial strains, e.g. *Escherichia coli* and *Bacillus cereus*[15]. Its low toxicity together with its pleasant taste and smell suggests its use as a food additive to prevent bacterial

contamination. It causes damages to the cell membrane of *Pseudomonas aeruginosa* and unlike other terpenes, inhibits the proliferation of this germ.[16]

It has been reported that essential oil tested for its major phenolic compounds (Thymol and Carvocrol) showed good antimicrobial activity against the main group of carcinogenic bacteria and *C. albicans* as well.[17] Plants essential oil and their main compounds show antimicrobial activity against a wide range of microorganisms including antibiotic resistant bacteria and fungi. Carvocrol is a biological compound naturally present in plants such as oregano, which has well-known antibacterial,[18] antifungal,[19] insecticidal [20], anti parasitic as well as anti toxigenic effect. It has been shown to have a broad spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria. Trans-Caryophyllene exhibited antibacterial and antifungal activities. The activity increased with increasing concentrations of the essential oil.[21]

In the present investigation, we have identified the Carvocrol as one of the major compounds present in the essential oil of *Wedelia chinensis* this could be the reason for its significant antimicrobial activity observed in our study.

Carrageenan, Egg-albumin and Xylene induced inflammation in rats represent the classical model of oedema formation which is helpful in designing a non-steroidal anti-inflammatory drugs and selective COX - 2 inhibitors. Many studies showed that the COX - 2 mediated increases in prostaglandin E_2 production in the central nervous system (CNS) contributes to the severity of inflammation and pain response. The use of essential oils in therapeutics is increasing drastically due to bioactive compounds having health benefit activity against microbes, inflammation and oxidation. It activates PPAR and suppresses COX-2 inflammation.[22]

Plants essential oil of different families has been tested earlier in different animal model of inflammation for the development of new preventive and curative remedies. In our present study the anti-inflammatory activity exhibited by the essential oil was found to be more or less same as compared with standard anti-inflammatory drug, Diclofenac. The time taken for the reduction of inflammation is more or less equal when compared with the standard drug. The anti-inflammatory activity exhibited by the essential oil may be due to the presence of Carvocrol and other compounds in the plants oil because Carvocrol has already been proved as anti inflammatory drug by its role in activating the PPAR and Suppressing the Cox – 2 inflammation and Trans – Caryophyllene were effective in reducing platelet activating factor, bradykinin and ovoalbumin – induced mouse paw odema.[23]

From the above results from the detailed study, it shows that the essential oil of *Wedelia chinensis* possesses a significant role in inhibiting the bacterial and fungal growth and plays role in reducing the inflammation in different models of inflammatory studies. All these effects may be due to the presence of Carvocrol and Trans-Caryophyllene as their major compounds.

CONCLUSION

To conclude our study the antimicrobial and anti-inflammatory activity of *Wedelia chinensis* essential oil is attributed by the combination of many major compounds but the major compounds such as Carvocrol and Trans – Caryophyllene. It is also interesting to note that these above mentioned activities is due to its cocktail of compounds, including some of the trace elements, not all of which have been identified in this study. On the basis of our results, this essential oil may be used as alternative for food, cosmetics and medicine. In addition to their use for food and cosmetics, its potential for the treatment of acne and cancer has to be explored in the future.

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