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Research Article

BIOACTIVE EVALUATION OF THE ESSENTIAL OIL OF *PLECTRANTHUS AMBOINICUS* BY GC-MS ANALYSIS AND ITS ROLE AS A DRUG FOR MICROBIAL INFECTIONS AND INFLAMMATION

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ABSTRACT

The present study is focused on the evaluation of the Anti-Microbial and Anti-Inflammatory activity of extracted essential oil of *Plectranthus amboinicus* in the experimental animal models. Initial studies on the determination of the Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) have been carried out and a minimal dose of finally 50µg/ml was found to have activity against the selected bacterial and fungal cultures. Then the anti-microbial study was carried out with 50µg/ml and the result obtained was compared with the respective standard antibiotic discs such as Norfloxacin, Cefepime and Gatifloxacin for Bacteria and Fluconozole for Fungi. The essential oil of *Plectranthus amboinicus* showed a promising activity against all the selected bacteria and fungi. The Anti-inflammatory activity of the essential oil was determined by inducing rat with inflammatory agents like Carrageenan, Egg-albumin and Xylene in the respective inflammatory sites (paw and ears). The site of inflammation was measured using Vernier Caliper. The results obtained for anti-inflammatory activity of the essential oil of *Plectranthus amboinicus* was compared with the standard Anti-inflammatory drug, Diclofenac Sodium. The inflammation was found to reduce to normal in essential oil treated rat similar to the Diclofenac does. The bioactive compound evaluation of the essential oil was carried out using GC-MS and the major compounds present in the essential oil was identified as Carvocrol -14%, Thymol – 18%, Cis –Caryophyllene, t-Caryophyllene, p-cymene -10%. A detailed study on each of these bioactive compound isolate may help to develop an effective drug formulation.

Keywords: Thymol, Caryophyllene, Xylene, *Plectranthus amboinicus*, Norfloxacin.

INTRODUCTION

The world health organization (WHO) estimated that 80% of the population of developing countries depend on traditional medicines mostly plant drugs for their primary health care needs. Also, modern pharmacopoeia still contains at least 25% of drug derived from plants and many others are synthetic analogous build on prototype compounds isolated from medicinal plants. They are used for wide range of health related applications from common cold to memory improvement and treatment of poisonous snake bites, enhancements of general immunity etc., India is the one of the major countries inhabited by a large number of tribal communities who possess precious and unique knowledge about the use of wild plants for treating human ailments.[1]

Plectranthus amboinicus is a tender fleshy perennial plant in the family *Lamiaceae* with oregano like flavor and odor, reported for many traditional uses, especially for the treatment of cough, sore throat and other nasal congestion. It is also used for a range of other problems such as infection, rheumatism and flatulence.[2]

Pharmacological activities of *Plectranthus amboinicus* have been investigated by different groups of researchers which include ethno botanical use of the plant.[3] *Plectranthus amboinicus* also has anti-tumor and cytotoxic activities.[4, 5, and 6]. In eastern Cuba it is used as herbal mixture as a traditional medicine for treating catarrhal infections.[7] Application of bruised leaf on burns is also reported, the leaf extract shows regulatory influence on calcium oxalate stone formation in experimental rats. Researchers have also proved that the leaves of *Plectranthus amboinicus* are used to expel kidney stone.[8] Our investigation in the research is to find out the antimicrobial and in vivo anti-inflammatory activity of the essential oil of *Plectranthus amboinicus.* As this plant is rich sources of numerous biologically active compounds, we have identified the major bioactive compounds by GC-MS.

MATERIALS AND METHODS

Plant Material Collection and Authentication of plants

Fresh leaves of the selected plant *Plectranthus amboinicus* 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 *Plectranthus amboinicus*

Extraction of essential oil from *Plectranthus amboinicus* 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, is then collected and dried over anhydrous sodium sulphate and stored in vial at low temperature until analysis.[9]

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 and it was 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 and were collected to about 1 ml and it was serially diluted from 10^{-1} to 10^{-6}. 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) of the essential oil of *Plectranthus amboinicus* over bacterial strains is determined by testing with different concentration. A 100µl of the inoculum, initially adjusted to 10^{-6} CFU/ml, was spread onto 20 ml Mueller–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. [10] 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.

Determination of Minimum Fungicidal Concentration (MFC)

The MFC was determined by incorporating various concentration of essential oil such as 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.[11] The potato dextrose broth with 1 ml of adjusted spore suspension and standard antibiotic discs served as positive control and PDA broth with 50% of ethanol served as negative control. The tubes, which showed no visible growth after three days of incubation, served as MFC of essential oil and incubated at 28^{0}C for 3 days. Tests were carried out in triplicate.

Antibacterial Screening

Disc diffusion method

The disc diffusion method was employed for the determination of anti - bacterial activities of the essential oil. Paper discs (6 mm diameter) were impregnated with 50 µg/ml of the oil dissolved in 50% Ethanol final to a 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*) [10]. 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 anti - fungal activities of the essential oil. Paper discs (6 mm diameter) were impregnated with 50 µg/ml of the essential 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*). [10] PDA plates, with 50% ethanol essential oil were used as negative control and treated with antibiotic disc (Fluconozole 25mcg) were used as positive control. After incubation at 25 ± 2°C for 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 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 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 100 µg/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**.** For the further study 50µg animal doses were selected.[12]

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 *Plectranthus amboinicus* + 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 *Plectranthus amboinicus* 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.[13]

Carrageenan - Induced paw inflammation in Swiss Albino Rats

Anti-inflammatory activity of *Plectranthus amboinicus* 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 *Plectranthus amboinicus 40* µg/kg i.p.30 min prior to Carrageenan injection. The paw volume of the Rats was measured using Vernier caliper prior and after every 1 hour from 1st - 24th hour of Carrageenan injection.[14]

Egg - albumin- induced inflammation in Swiss Albino Rats

Swiss Albino Rats (250-300)g of either sex randomized into 4 different groups of 5 rats each for the experiment. Animals were treated Intra peritoneally with the essential oil of *Plectranthus amboinicus* (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. 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. The inflammation was assessed by measuring hind paw with using Vernier caliper.[15]

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 in INDIAN INSTITUTE OF SPICES RESEARCH (IISR)-CALICUT-KERALA- [PMT/IISR/28(13)09]. Using CARBOWAX capillary column and Helium as carrier gas to quantify the major phytochemicals present in the essential oil. The identification was based on comparison of their mass spectra and retention indices.[16]

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.

Table1: Minimum Inhibitory Concentration (MIC) of Essential oil of *Plectranthus amboinicus* on selected Bacterial species

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

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

S. No.	Cultures and Dilution used(10^{-6})	Zone of inhibition (mm) of *Plectranthus amboinicus*				
		50 µg/ml	Positive Control			Negative Control
			Norfloxacin 10µg	Cefepime 30µg	Gatifloxacin 5µg	
1	*Escherichia coli*	25±1.00	22.3±1.15	22.3±1.155	25.66±0.577	-
2	*Staphylococcus aureus*	25.6±0.57	25.33±0.57	16.66±1.528	13.66±1.528	-
3	*Pseudomonas aeruginosa*	31.6±1.528	34.6±0.57	27±26.66	26.6±4.163	-
4	*Streptococcus pneumoniae*	23.3±2.88	26.3±0.57	21.3±1.528	25±2.646	-

Table3: Minimum Fungicidal Concentration (MFC) of Essential oil of *Plectranthus amboinicus* 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 *Plectranthus amboinicus* on selected fungal species

S. No.	Cultures and Dilution used(10^{-4})	Zone of inhibition (mm) of Plectranthus amboinicus		
		50 µg/ml	Positive Control Fluconozole 25 µg	Negative Control (50% Ethanol)
1	*Candida albicans*	18.6±4.72	24.3±0.577	-
2	*Candida parapsilosis*	18.6±4.04	17.6±2.51	-
3	*Candida tropicalis*	18.3±5.50	21±4.00	-

Table 5: Acute Toxicity Study of Essential oil of *Plectranthus amboinicus*

S. No.	Concentration of the Drug (µg/kg i.p.,)	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 *Plectranthus amboinicus* on Carrageenan Induced inflammation in Swiss Albino Rats

Treatment	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	1.17 **(47)** ±0.02	1.17 **(47)** ±0.02	1.14 **(45)** ±0.01	1.14 **(45)** ±0.01	1.12 **(44)** ±0.01	1.11 **(43)** ±0.01
Group II	0.63 ±0.01	1.17 **(47)** ±0.02	1.14 **(45)** ±0.01	1.11 **(44)** ±0.01	1.11 **(44)** ±0.01	1.11 **(44)** ±0.01	1.11 **(43)** ±0.01
Group III	0.64 ±0.01	1.13 **(45)** ±0.03	1.03 **(39)** ±0.11	0.87 **(28)** ±0.05	0.84 **(26)** ±0.03	0.81 **(25)** ±0.02	0.79**(21)** ±0.01
Group IV	0.66 ±0.01	1.10 **(43)** ±0.05	0.89 **(30)** ±0.05	0.87 **(29)** ±0.03	0.86 **(28)** ±0.03	0.84 **(26)** ±0.02	0.81 **(25)** ±0.01

Table 7: Effect of Essential oil of *Plectranthus amboinicus* on Egg- Albumin Induced inflammation in Swiss Albino Rats

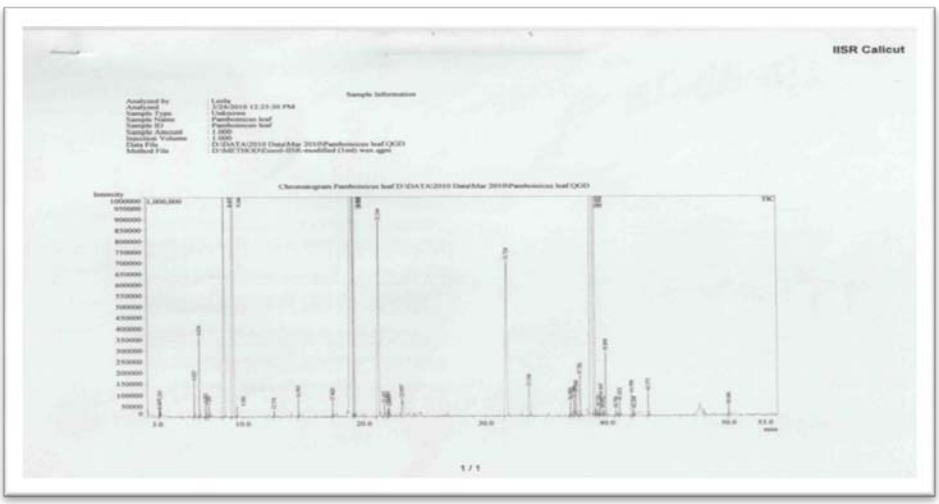
Treatment	Time Intervals (in hours) Readings (in cm) (in %)						
	0th Hour	1st Hour	3rd Hour	6th Hour	9th Hour	12th Hour	24th Hour
Group I	0.69 ±0.01	1.17 **(47)** ±0.01	1.17 **(47)** ±0.01	1.17 **(47)** ±0.01	1.14 **(45)** ±0.01	1.14 **(45)** ±0.01	1.12 **(44)** ±0.01
Group II	0.65 ±0.01	1.17 **(47)** ±0.01	1.17 **(47)** ±0.01	1.14 **(45)** ±0.01	1.12 **(44)** ±0.01	1.08 **(43)** ±0.13	1.08 **(43)** ±0.13
Group III	0.63 ±0.01	1.17 **(47)** ±0.01	1.08 **(43)** ±0.13	0.99 **(37)** ±0.06	0.93 **(33)** ±0.04	0.82 **(24)** ±0.02	0.78 **(21)** ±0.02
Group IV	0.65 ±0.01	1.17 **(47)** ±0.01	0.96 **(35)** ±0.02	0.96 **(35)** ±0.02	0.82 **(24)** ±0.008	0.82 **(23)** ±0.01	0.81 **(23)** ±0.01

Table 8: Effect of Essential oil of *Plectranthus amboinicus* on Xylene Induced inflammation in Swiss Albino Rats

Treatment	Weight of Right ear (g)	Weight of Left ear (g)	Increase in ear weight (g)	% Increase in ear weight	% Inhibition
Group I	0.12±0.008	0.18±0.035	0.06	50	-
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	41.2
Group IV	0.16±0.018	0.20±0.025	0.04	30	40

Table 9: GC - MS Peak Report of Essential oil of *Plectranthus amboinicus*

Peak S. No.	Retention Time	Initial Time	Final Time	Area	Area%	Height	Height%	Area/ Height	Name of the Compound
1	3.167	3.142	3.192	37816	0.04	23126	0.17	1.63	
2	3.215	3.192	3.250	103985	0.10	644636	0.47	1.61	
3	**6.025**	**5.942**	**6.083**	**519929**	**0.48**	**163595**	**1.19**	**3.17**	***β- myrcene***
4	**6.434**	**6.350**	**6.492**	**1195335**	**1.11**	**367953**	**2.67**	**3.24**	***α- terpinene***
5	6.951	6.892	7.000	187301	0.17	65040	0.47	2.87	
6	7.205	7.150	7.267	167576	0.16	53122	0.39	3.15	
7	**8.497**	**8.283**	**8.525**	**7052727**	**6.58**	**1080705**	**7.83**	**6.52**	***γ- terpinene***
8	**9.246**	**9.017**	**9.275**	**11613095**	**10.83**	**1496414**	**10.85**	**7.76**	***P-cymene***
9	9.509	9.467	9.558	104894	0.10	48464	0.35	2.16	
10	12.576	12.533	12.617	79707	0.07	33527	0.24	2.37	
11	14.593	14.533	14.642	208035	0.19	860544	0.62	2.41	
12	17.420	17.367	17.475	204023	0.19	73368	0.53	2.78	
13	**19.063**	**18.658**	**19.083**	**19370542**	**18.06**	**1493690**	**10.83**	**12.96**	***Cis- Caryophyllene***
14	**19.125**	**19.083**	**19.158**	**5420409**	**5.05**	**1255582**	**9.10**	**4.31**	***Trans Caryophyllene***
15	**19.195**	**19.158**	**19.267**	**5679118**	**5.30**	**1236523**	**8.96**	**4.59**	***Trans Caryophyllene***
16	**21.244**	**20.983**	**21.308**	**7313588**	**6.82**	**887597**	**6.43**	**8.23**	***α humulene***
17	21.657	21.583	21.767	240104	0.22	57586	0.42	4.16	
18	21.960	21.908	22.008	98197	0.09	32116	0,23	3.05	
19	22.053	22.008	22.125	122471	0.11	39207	0.28	3.12	
20	23.097	23.025	23.158	212666	0.20	52445	0.38	4.05	
21	**31.724**	**31.475**	**31.817**	**6213111**	**5.79**	**710896**	**5.15**	**8.73**	***Aromadendrene***
22	33.558	33.417	33.675	999522	0.93	131655	0.95	7.59	
23	36.989	36.908	37.067	318744	0.30	70300	0.51	4.53	
24	37.286	37.208	37.350	287522	0.27	74521	0.54	3.85	
25	37.440	37.350	37.517	416408	0.39	101193	0.73	4.11	
26	**37.781**	**37.683**	**37.883**	**844968**	**0.79**	**866432**	**1.35**	**4.53**	***Thymol***
27	**38.690**	**38.492**	**38.842**	**19401104**	**18.09**	**1350641**	**9.79**	**14,36**	***Thymol***
28	**38.994**	**38.842**	**39.042**	**15239984**	**14.21**	**1710851**	**12.40**	**8.90**	***Carvocrol***
29	39.092	39.050	39.150	92591	0.09	29222	0.21	3.16	
30	39.320	39.275	39.375	97919	0.09	35523	0.26	2.75	
31	39.507	39.408	39.625	463249	0.43	89315	0.65	5.18	
32	39.890	39.775	39.975	1239327	1.16	290525	2.11	4.26	
33	40.706	40.608	40.775	170325	0.16	34625	0.25	4.91	
34	41.032	40.958	41.108	274334	0.26	69057	0.50	3.97	
35	41.998	41.908	42.083	464876	0.43	104413	0.76	4.45	
36	42.208	42.142	42.267	123347	0.12	35149	0.25	3.50	
37	43.373	43.292	43.458	482056	0.45	113514	0.82	4.24	
38	50.000	49.933	50.075	169310	0.16	46601	0.34	3.63	



Graph 5: Results of GC and GC-MS Analysis of Essential Oil of *Plectranthus amboinicus*

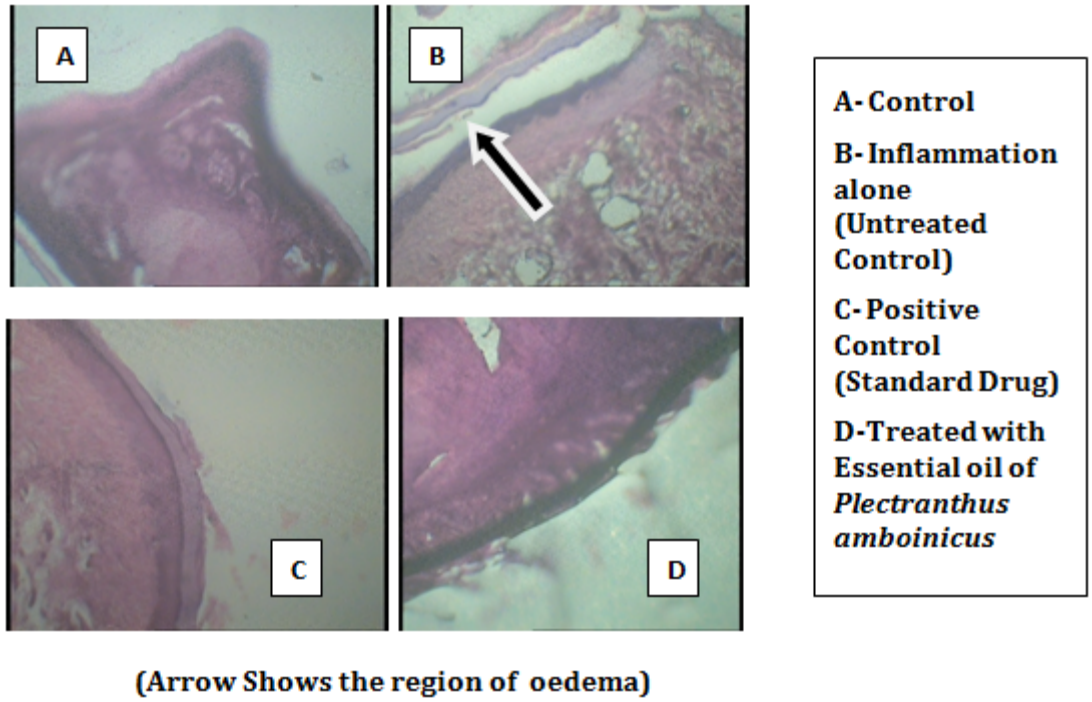


Fig. 1: Histopathological Studies of Inflammation in Legs of Swiss Albino Rats

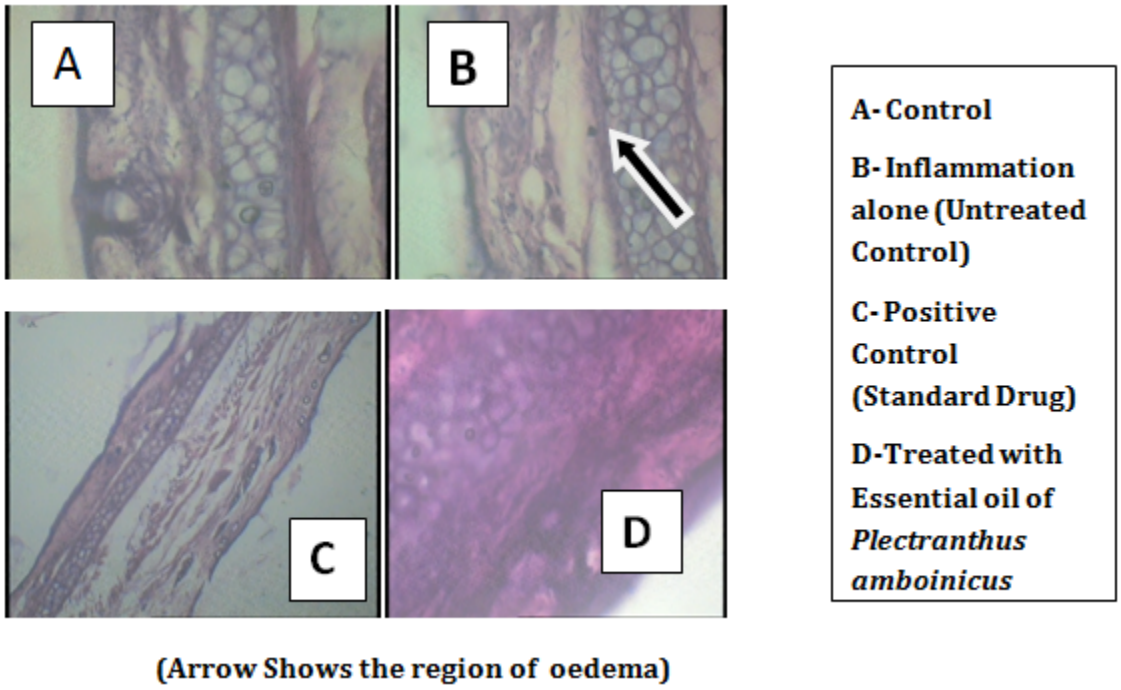


Fig. 2: Histopathological Studies of Inflammation in Ears of Swiss Albino Rats

RESULT

The bioactive evaluation of essential oil of *Plectranthus amboinicus* has been done by extracting the oil from fresh leaves using Clevenger apparatus by Hydro – distillation method. By employing this method the yield of oil was 1ml/200gms of the fresh leaves.

Table1 shows the Minimal Inhibitory Concentration (MIC) of the essential oil against the bacterial strains used for the study. Out of the different concentrations 25, 50, 75 and 100µg/ml used to determine the MIC of the essential oil, we found that 50 µg/ml is the minimal dose to inhibit the growth of the microorganisms. The zone of inhibition has been studied for the anti-microbial activity of the essential oil of *Plectranthus amboinicus* against the test bacteria. Table 2 shows the Zone of Inhibition (ZOI) of the essential oil in which the zone was found to be 25mm for *Escherichia coli*, which is more or less equal to the standard antibiotic discs Norofloxacin - 22.3mm, Cefepime - 22.3mm and Gatifloxacin of 25.6mm. The ZOI of essential oil against *Staphylococcus aureus* and *Pseudomonas*

aeruginosa was found to be the maximum as 31.6mm which is higher than that obtained for standard antibiotic discs Cefepime 27mm and Gatifloxacin26.6mm This shows that the essential oil of *Plectranthus amboinicus* have a significant anti-bacterial activity.

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

Table 4 shows the Anti-fungal activity of the essential oil in terms of zone of Inhibition against the selected fungal species when compared with the standard anti-fungal disc Fluconozole. The result shows that *Candida albicans* is more susceptible to essential oil with the inhibitory zone of 15mm which is more or less equal in comparison with positive control 17mm. Next to *C.albicans,* the fungi *C.parapsilosis* and *C.tropicalis* were also susceptible to the essential oil with the zone of 13.6 and 12mm respectively. The results indicate that the essential oil of *Plectranthus amboinicus* is having a significant antifungal activity.

The acute toxicity study of essential oil of *Plectranthus amboinicus* shows that up to 50µg/kg there was no sign of toxicity as shown in table 5. From this observation, the dose (40 µg/kg) below 50µg/ml was used for in-vivo studies. Table 6 shows the activity of essential oil of *Plectranthus amboinicus* on Carrageenan induced inflammation in rats. The results infers that the negative control showed a delayed decrease in the inflammation whereas the positive control Diclofenac, the standard anti-inflammatory drug shows the faster decrease in the inflammation with time lapse and the essential oil of *Plectranthus* also shows the gradual decrease in the inflammation with respect to time interval. Similar result was obtained in Egg-albumin induced inflammation in rats in which the essential oil showed a more or less equal activity with Diclofenac in reducing the inflammation within the stipulated time period.

Table 8 infers the activity of essential oil of *Plectranthus amboinicus* on Xylene induced inflammation in rats. The table shows that the standard drug Diclofenac has the higher percentage inhibition (56.8%) and the essential oil shows a moderate inhibitory activity (34%), showing its anti-inflammatory activity.

Table 9 shows the result for the GC-MS analysis of the essential oil of *Plectranthus amboinicus* containing 15 compounds. The major compounds includes Carvocrol -14%, Thymol – 18%, Cis – Caryophyllene, t-Caryophyllene, p-cymene -10% with 96% equal comparison with the Willey and NBS library.

DISCUSSION

Essential oil extraction was carried out by hydro distillation method from the plant material for the study. It is a modernized special type of distillation or separation process for temperature sensitive materials like oils, resins, and hydrocarbons etc., which are insoluble in water and may decompose at their boiling point. Quantitative analysis of essential oil is done by using GC-MS. From this, the purity of the compounds can be identified in the essential oil of the plant.[17] In the present investigation, the result of GC – MS analysis reveals the presence of 11compounds, out of which Carvocrol -14%, Thymol – 18%, Cis –Caryophyllene, t-Caryophyllene, p-cymene -10% were found to be the major compounds.

Earlier researchers have highlighted that the essential oils having high concentration of Thymol and Carvocrol usually inhibit gram - positive more than gram – negative pathogenic bacteria.[18] Apart from the anti-bacterial and anti-fungal activities, essential oils have also been reported to posses interesting anti-viral activities as an alternative to synthetic anti-viral drugs.[19] The essential oil containing Carvocrol and Thymol has been demonstrated to have Virucidal properties with the advantage of low toxicity.[20] Cymene, a biological precursor of Carvocrol, by itself does not have anti-microbial activity but it can enhance the inhibitory effect of Carvocrol when it is used together.[21] However, anti-microbial activity of Carvocrol and cymene against drug resistant organisms has never been reported. Due to the smell and taste of Carvocrol at high concentrations, Carvocrol was combined with cymene, a natural anti-microbial compound with a similar structure. It has been reported that the phenolic Monoterpenes and Carvocrol is able to disintegrate the outer membrane of Gram-negative bacteria, releasing Lipo polysaccharides (LPS) and increasing the permeability of the cytoplasmic membrane to Adenosine Tri Phosphate (ATP).[22]

The present study infers that there is a significant decrease in the bacterial and fungal growth by the essential oil of *Plectranthus amboinicus* which may be because of the presence of Carvocrol, Thymol and p-Cymene as their major compounds. The zones obtained for the essential oil was found to be more or less similar to the zones obtained for the standard antibiotic discs. This implies that the essential is having an efficient inhibitory activity towards the Multi Drug Resistant bacteria and fungi that we used for the study.

In Indian system of medicine, certain herbs are claimed to provide relief from pain and inflammation. Inflammation is a complex process and Relative Oxygen Species plays an important role in the pathogenesis of inflammatory diseases.[23] Inflammation is a process of host response to tissue injury and it finally leads to the restoration of a normal tissue structure and function. Acute inflammation is a limited beneficial process, particularly in response to infectious pathogens, but chronic inflammation is an undesirable persistent phenomenon that leads to the development of inflammatory oriented diseases. Prolonged inflammation contributes to the pathogenesis of many inflammatory diseases like metabolic disease, arthrosclerosis, obesity, cardiovascular disease, rheumatoid arthritis and even cancer.[24]

Carrageenan and egg-albumin induced paw inflammation has been followed by many researchers and is a phylogistic tool for investigating anti-inflammatory agents. There are mainly biphasic effects in all the models of inflammation. The early hyperemia results from the release of histamine and serotonin and the delayed phase of Carrageenan and egg – albumin induced oedema results mainly from the potentiating effects of bradykinin on mediator release, and of prostaglandins producing edema after the mobilization of leukocytes.

An earlier study shows that the ethanolic extract of *Plectranthus amboinicus* possesses nephrotoxicity effects against acetaminophen-induced nephrotoxicity and strong diuretics effect in rats.[25] However, the therapeutic potential of *Plectranthus amboinicus* for inflammatory diseases remains unclear.

The present investigation on the essential oil of *Plectranthus amboinicus* for its anti-inflammatory activity using Carrageenan, egg – albumin and xylene models reveals that there is a reduction in the inflammation in time and dosage dependent manner. The essential oil of our study plant shows a significant decrease in the inflammation when compared with the standard drug, Diclofenac with more or less equal measure.

CONCLUSION

To conclude the study result, the essential oil of *Plectranthus amboinicus* showed a significant anti-microbial and anti-inflammatory activity. Therefore, the essential oil has inhibited both acute and chronic inflammatory process, similar to COX inhibitors, but it does not induce any side effects during the treatment of anti-inflammatory studies. It is strongly suggested that the pharmacological activity of the essential oil of *Plectranthus amboinicus* may be because of the presence of major compounds Carvocrol and Thymol and hence a detailed study on this plant is needed for further drug formulations in future.

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