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Research Article

# ANTIFUNGAL, ANTI-INFLAMMATORY AND GC – MS ANALYSIS OF METHANOLIC EXTRACT OF *PLECTRANTHUS AMBOINICUS* LEAF

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**ABSTRACT**

In the present study, the antifungal, anti-inflammatory and GC-MS analysis of the methanol extract of Plectranthus amboinicus leaf was investigated. The various concentrations of extracts were investigated for its antifungal with the positive and negative controls and anti inflammatory activity were assessed in Balb/c mice models. The antifungal activity of methanolic extract of Plectranthus amboinicus was assessed by analyzing the MFC of the extract and from the results obtained from the MFC of the extract 150, 250, 500 µg/ml were found to be having higher inhibition over the fungal species selected for the study and hence these above mentioned concentrations were taken for the confirmation of the antifungal activity by zone of inhibition method and the result reveals that the extract shows significant antifungal activity with a P value of [P<0.05-0.01] when compared with the positive control the standard antifungal drug Fluconozole. The methanolic extract of Plectranthus amboinicus was given intraperitoneally (i.p.) in the form of suspension in 2% gum acacia in two different doses, 250 and 500 mg/kg body weight. The result showed that the plant has significant reduction [P<0.05-0.01] in inflammation in both the concentration of the extracts but 500mg/kg gives the faster reduction in the inflammation than the 250mg/kg when compared that with the standard drug, Diclofenac. The GC-MS results were performed to find out the major active phytochemical compounds in the essential oil of the fresh leaves of the plant. These results indicate that the extracts possess antifungal and anti-inflammatory properties.

**Keywords:** Plectranthus amboinicus, Diclofenac, Carrageenan – induced oedema, GC – MS, anti – inflammatory.

## INTRODUCTION

Plants, owing to its medicinal value have continued to play a dominant role in the maintenance of human health since ancient times. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [1]. Over 50% of all modern clinical drugs are of natural product origin [2]. Turkish people have a tradition of using a number of plant species for the treatment of infectious diseases and various ailments [3]. Traditional and folklore medicines play important role in health services around the globe. About three quarter of the world's population rely on plant and plant extracts for healthcare. India has an extensive forest cover, enriched with plant diversity.

The subcontinent is rich in medicinal plants and is one of the richest countries in terms of genetic diversity of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover the agro climatic conditions are conducive for introducing and domesticating new exotic plant varieties [4]. Several plants have been used in folklore medicine. The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare. *Plectranthus amboinicus* is a succulent herb and has the typical four-cornered stem of the Lamiaceae family. The leaves are very thick and succulent, grayish green and hairy. The plant grows to a length of around 50 cm. The leaves are highly aromatic with a strong flavor of mixed herbs. The herb grows easily in a well-drained, semi-shaded position. It is frost tender and grows well in sub-tropical and tropical locations, but will also do well in cooler climates if grown in a pot and brought indoors, or moved to a warm sheltered position in winter.

The leaves have also had many traditional medicinal uses, especially for the treatment of coughs, sore throats and nasal congestion, and also for a range of other problems such as infections, rheumatism and flatulence. In Indonesia *Plectranthus amboinicus* is a traditional food used in soup to stimulate lactation for the month or so following childbirth. The herb is also used as a substitute for oregano in the food trade and food labeled "oregano-flavored" may well contain this herb. The multidrug resistant strain of many microorganisms has revealed exploration of alternative antimicrobial agent. Medicinal plants have become the focus of intense study in terms of validation of their traditional uses through the determination of their actual pharmacological effects. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often adulterated and possess side effects. Inflammation is a local response of living mammalian tissues to the injury. It is a body defense mechanism reacting in order to eliminate or limit the spread of injurious agents.

There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Odema formation, leukocyte infiltration and granuloma formation represent such components of inflammation. Odema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and or the mediators that increase blood flow. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs possess well known toxic and side effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. Exploring the healing power of plants is an ancient concept. For centuries people have been trying to alleviate and treat disease with different plant extracts and formulations [8].

It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Plants still represent a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs [7]. Gas chromatography-mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GC/MS can also be used in airport security to detect substances in luggage or on human beings. However, fewer reports are available with respect to the pharmacological properties of the plant. Keeping this in view, the present study has been undertaken to investigate the anti - fungal and anti-inflammatory effects of methanol extract of *Plectranthus amboinicus* (MEPA) in standard animal models.

## MATERIALS AND METHODS

### Plant material

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

### Preparation of the plant Extract

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

### Anti-fungal activity

### Fungal inoculum preparation

The fungal cultures *Candida albicans, Aspergillus fumigates, Candida tropicalis, Aspergillus flavus and Aspergillus niger* were obtained from Dr. N. G. P. Arts and Science College and Tamilnadu Agriculture University (TNAU), Coimbatore, Tamil Nadu. These fungal cultures were maintained in potato dextrose agar plates and slants and was further sub cultured before use. The mother inoculum was maintained at $37^0$C for about 48 to 72 hours. The fungal spores were scooped out by adding 1ml of sterile distilled water. The fungal spores were collected to about 1 ml and it was serially diluted from $10^{-1}$ to $10^{-6}$ and plating was made using $10^{-4}$ dilution.

### Determination of Minimum Fungicidal Concentration (MFC)

MFC was determined according to agar dilution method [21, 22]. Various concentrations (50, 100, 150, 200, 250, 300, 350, 400, 450, 500 mg/ml) of each extracts were prepared in 10 cm experimental tubes containing PDA broth. Each tube contains 9 ml of PDA and was sterilized by autoclaving. Upon cooling, 1 ml of each extract concentration was added into the respective tubes. The mixture of PDA and extracts were poured into plates aseptically in a laminar flow cabinet. Upon solidification of the agar medium, 2 µl of adjusted spore suspension were added to each plate by micropipette and incubated at 28° C for three days. The PDA without any herbal extract served as control. The MFC was regarded as the lowest concentration of the extract that prevented the growth of any fungal colony on the solid medium.

### Agar well diffusion method

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

### Experimental animals

BALB/c 25-30g, were grouped and housed in polyacrylic cages (6 per group and cage) and maintained under standard laboratory conditions (temperature 24-28 ºC, RH – 60-70% and 12 hours light and dark cycles). They are housed in cages for at least one week before starting experiments. They were fed commercial mice feed (Sri Sai Durga Feeds and Food, Bangalore) and boiled water, ad libitum. All the experiments involving animals were done according to standard protocols from NIH guidelines, after getting approval from the university Animal Ethics Committee. All animals were fasted overnight before commencing each experiment.

### Acute toxicity study

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

### Anti-Inflammatory activity

### Xylene induced ear oedema

*BALB/c 25-30g* was divided into four groups of six animals each. Animals were treated intra peritoneally with the extract (250 and 500 mg/kg), Diclofenac (100 mg/kg) and 0.1ml of 2% gum acacia. Thirty minutes later, oedema was induced in each mouse group by applying a drop of xylene to the inner surface of the right ear. After 15 min, the animals were sacrificed under ether anesthesia and both ears were cut off, sized and weighed [11]. The anti-inflammatory activity was expressed as the percentage inhibition of oedema in the treated mice in comparison with the control mice. The difference between ear weights was taken as the oedema induced by xylene.

### Carrageenan - induced paw oedema in mice

Anti-inflammatory activity of *Plectranthus amboinicus* was assessed by Carrageenan induced paw oedema method [12]. Mice were divided into 4 groups (6 animals in each group). Animals of all the groups were injected with 0.1 ml of 1% Carrageenan in 0.9% normal saline, under the plantar aponeurosis of the right hind paw. Group I animals (Carrageenan control) received 0.1ml of 2% gum acacia i.p., 30 min prior to Carrageenan injection. Group II, the standard reference group was given i.p., an aqueous solution of Diclofenac (100 mg/kg) 30 min prior to Carrageenan injection. Group III and Group IV received i.p., 200 and 400 mg/kg of *Plectranthus amboinicus* methanolic extract suspension in 2% gum acacia, 30 min prior to Carrageenan injection, respectively. The paw volume of the mice was measured using Vernier calliper just prior to and after ($0^{th}$ hour, every 30min between $1^{st}$ -$8^{th}$ hour, $12^{th}$ and $24^{th}$ hour) Carrageenan injection.

### Egg-albumin- induced inflammation in Mice

*BALB/c 25-30g* of either sex randomized into 4 different groups of 6 mice each were used for the experiment. The leaf extract (250 and 500 mg/kg i.p) and Diclofenac (100mg/kg orally) were administered to mice 1 hr before the induction of inflammation. Control group received 0.1ml of 2% gum acacia i.p. Inflammation was induced in mice by the injection of 0.1 ml of fresh egg-albumin into the sub planar tissue of the right hind paw. The linear circumference of the injected paw was measured before and 0.5, 1, 2, 3, 4 and 5hrs after the administration of the phlogistic agent. Oedema (inflammation) was assessed as the difference in paw circumference between the control and 0.5, 1, 2, 3, 4 and 5hrs after the administration of the phlogistic agent [13]. All the animals were fasted for 24 hours before the commencement of the experiment. The average (mean) edema was assessed by measuring with Vernier calipers.

### Formalin induced oedema in Mice

Anti-inflammatory activity was evaluated by formalin induced paw oedema method. *BALB/c 25-30g* of either sex was divided into 4 groups (6 animals in each group). Animals of all the groups were injected with 0.1 ml of 1% formalin in 0.9% normal saline, under the plantar aponeurosis of the right hind paw. Group I animals (formalin control) received 0.1ml of 2% gum acacia i.p., 30 min prior

to formalin injection. Group II, the standard reference group was given i.p., an aqueous solution of Diclofenac (100 mg/kg), 30 min prior to formalin injection. Group III and Group IV received i.p., 250 and 500 mg/kg of *Plectranthus amboinicus* methanolic extract suspension in 2% gum acacia, 30 min prior to formalin injection. The paw volume of the mice was measured using Vernier calliper just before and after 3rd, 6th, 12th and 24th hour following formalin injection. The percentage inhibition of the oedema was calculated for each with respect to the vehicle treated control group [14].

**Formaldehyde induced oedema in Mice**

*BALB/c* weighing between 25-30 g was randomly grouped in four groups with 6 mice each. On the 0th day, the basal paw volume of left hind paw of each animal was measured using Vernier calliper. Dosing with standard drug, Diclofenac sodium and extracts was started on same day and continued for 10 days. 0.1 ml of 2 % v/v formaldehyde in normal saline was injected into the sub-plantar region of the left hind paw. Group I served as control, Group II Diclofenac Sodium (standard drug) treated and Group III & IV as plant extract 250, 500 mg/kg respectively. Paw volume of injected paw was measured on the 1st, 2nd, 3rd, 4th and 5th day [15].

**GC-MS analysis**

GC-MS analysis was performed in INDIAN INSTITUTE OF SPICES RESEARCH (IISR)-CALICUT-KERALA- [PMT/IISR/28(13)09].

Essential oils were extracted from *Plectranthus amboinicus* based on hydro distillation method for GC-MS analysis. GC-MS analysis was performed using SHIMADZU GC – MS QP 2010 using CARBOWAX capillary column and Helium as carrier gas to quantify the major Phytochemicals. 0.2µl of essential oil was injected in to the column at the flow rate of 1µl/minute. The injector was operated at $250^0$C and the oven temperature was programmed as follows; $60^0$C for 15minutes, then gradually increased to $280^0$C at 3minutes. The identification of components were based on comparison of their mass spectra with those of Wiley and NBS libraries and those described by Adams as well as comparison of their retention indices.

**Statistical analysis**

The calculation of the average antifungal activity of different concentration of the plant extract and anti – inflammation activity in the paw were based on the expression of numerical data as mean ± SD. The statistical significance between control and treated groups were analyzed using analysis of variance (ANOVA), where p<0.05*, p<0.01** and P<0.001*** were taken to be significant [16].

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

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

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

| S.NO | Cultures and Dilution used($10^{-4}$) | Zone of inhibition (mm) of *Plectranthus amboinicus* | | | | |
|---|---|---|---|---|---|---|
| | | 150 µg/ml | 250 µg/ml | 500 µg/ml | Positive control | Negative control |
| 1 | *Candida albicans* | 11.3** ±1.15 | 13.6** ±0.57 | 14.6** ±0.57 | 18.3 ±3.51 | 18.3 ±0.57 |
| 2 | *Aspergillus fumigatus* | 14.6* ±1.15 | 16*** ±1.73 | 15.3* ±0.57 | 19.6 ±2.08 | 17.6 ±0.57 |
| 3 | *Candida tropicalis* | 11.3** ±1.15 | 13.6** ±0.57 | 14* ±1.00 | 19 ±2.64 | 18 ±1.00 |
| 4 | *Aspergillus flavus* | 10.3* ±0.57 | 13.6** ±1.15 | 14.3** ±0.57 | 20 ±2.00 | 18.3 ±0.57 |
| 5 | *Aspergillus niger* | 11*** ±1.00 | 13* ±1.00 | 12*** ±1.00 | 22.3 ±1.52 | 19 ±1.00 |

µg/ml = Concentration of plant extract, Data are expressed as mean ±S.D. Significant at P<0.05*, P<0.01** and P<0.001***and Non significant (ns), when compared to control n=3(Triplicates) *(P.A. - Plectranthus amboinicus)*

**Table 3: Effect of *P.A.* leaf extract on Carrageenan Induced oedema in mice**

| Treatment dose (mg/kg) | Time intervals(in hours) readings (in mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 | 3.5 | 4 | 4.5 |
| Control (10ml/kg) | 0.24 ±0.01 | 0.47 ±0.02 | 0.47 ±0.02 | 0.46 ±0.02 | 0.46 ±0.02 | 0.45 ±0.01 | 0.45 ±0.01 | 0.42 ±0.01 | 0.42 ±0.01 | 0.41 ±0.01 |
| Standard drug (100mg/kg) | 0.25** ±0.01 | 0.41** ±0.02 | 0.43** ±0.01 | 0.43** ±0.02 | 0.42** ±0.01 | 0.39** ±0.01 | 0.38** ±0.02 | 0.37** ±0.01 | 0.37** ±0.01 | 0.36** ±0.02 |
| Extract (250 mg/kg) | 0.22** ±0.01 | 0.47ns ±0.01 | 0.46ns ±0.01 | 0.45ns ±0.01 | 0.45ns ±0.01 | 0.43** ±0.01 | 0.41** ±0.01 | 0.41ns ±0.01 | 0.40** ±0.01 | 0.39* ±0.01 |
| Extract (500mg/kg) | 0.23** ±0.01 | 0.46ns ±0.01 | 0.46ns ±0.01 | 0.45ns ±0.01 | 0.44** ±0.01 | 0.43** ±0.01 | 0.42* ±0.01 | 0.40ns ±0.01 | 0.39** ±0.01 | 0.38** ±0.01 |

| Treatment dose (mg/kg) | Time intervals(in hours) readings (in mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5 | 5.5 | 6 | 6.5 | 7 | 7.5 | 8 | 12 | 24 |
| Control (10ml/kg) | 0.39 ±0.01 | 0.38 ±0.01 | 0.35 ±0.01 | 0.34 ±0.01 | 0.34 ±0.01 | 0.33 ±0.01 | 0.32 ±0.01 | 0.31 ±0.01 | 0.31 ±0.01 |
| Standard drug (100mg/kg) | 0.35** ±0.02 | 0.34** ±0.01 | 0.33*** ±0.01 | 0.32** ±0.01 | 0.32ns ±0.01 | 0.31** ±0.01 | 0.30** ±0.02 | 0.29** ±0.01 | 0.27** ±0.01 |
| Extract (250 mg/kg) | 0.35* ±0.01 | 0.34** ±0.01 | 0.33** ±0.01 | 0.30** ±0.01 | 0.30** ±0.01 | 0.27** ±0.01 | 0.28** ±0.01 | 0.27** ±0.01 | 0.25** ±0.01 |
| Extract (500mg/kg) | 0.37** ±0.01 | 0.35** ±0.01 | 0.32** ±0.01 | 0.33ns ±0.01 | 0.29** ±0.01 | 0.29** ±0.01 | 0.26*** ±0.01 | 0.25** ±0.01 | 0.23** ±0.01 |

Data are expressed as mean ±S.D. Significant at P<0.05*, P<0.01** and P<0.001*** and Non significant (ns), when compared to control n=6. *(P.A. - Plectranthus amboinicus)*

**Table 4: Effect of *P.A.* leaf extract on Formaldehyde Induced oedema in mice**

| Treatment dose (mg/kg) | Time intervals(in days) readings (in mm) | | | | | |
|---|---|---|---|---|---|---|
| | 0th day | 1st day | 2nd day | 3rd day | 4th day | 5th day |
| Control (10ml/kg) | 0.34 ±0.01 | 0.56 ±0.01 | 0.55 ±0.01 | 0.54 ±0.01 | 0.53 ±0.03 | 0.51 ±0.02 |
| Standard drug (100mg/kg) | 0.25** ±0.01 | 0.54*** ±0.01 | 0.50** ±0.01 | 0.47** ±0.01 | 0.44** ±0.04 | 0.38** ±0.02 |
| Extract (250 mg/kg) | 0.25** ±0.01 | 0.55ns ±0.01 | 0.55* ±0.01 | 0.51** ±0.01 | 0.47** ±0.02 | 0.44** ±0.02 |
| Extract (500mg/kg) | 0.23** ±0.01 | 0.55** ±0.04 | 0.53** ±0.01 | 0.52ns ±0.02 | 0.46*** ±0.03 | 0.42** ±0.01 |

Data are expressed as mean ±S.D. Significant at P<0.05*, P<0.01** and P<0.001*** and Non significant (ns), when compared to control n=6. *(P.A. - Plectranthus amboinicus)*

**Table 5: Effect of *P.A.* leaf extract on Xylene Induced oedema in mice**

| Treatment dose (mg/kg) | Weight of Right ear (g) | Weight of Left ear (g) | Increase in ear weight (g) | % Increase in ear weight | % Inhibition |
|---|---|---|---|---|---|
| Control (10ml/kg) | 0.123 ±0.003 | 0.056 ±0.001 | 0.069 ±0.001 | 56.09 | - |
| Standard drug (100mg/kg) | 0.111** ±0.001 | 0.055ns ±0.001 | 0.056** ±0.001 | 50.45 | 49.54 |
| Extract (250 mg/kg) | 0.149*** ±0.025 | 0.054** ±0.001 | 0.095** ±0.002 | 63.75 | 36.24 |
| Extract (500mg/kg) | 0.153** ±0.002 | 0.052** ±0.001 | 0.101*** ±0.002 | 66.01 | 33.98 |

Data are expressed as mean ±S.D. Significant at P<0.05*, P<0.01** and P<0.001*** and Non significant (ns), when compared to control n=6. *(P.A. - Plectranthus amboinicus)*

**Table 6: Effect of *P.A.* leaf extract on Formalin Induced oedema in mice**

| Treatment dose (mg/kg) | Time intervals(in hours) readings (in mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | initial 0th hour | 1st hour | 3rd hour | 6th hour | 9th hour | 12th hour | 24th hour |
| **Control (10ml/kg)** | 0.38 ±0.01 | 0.54 ±0.01 | 0.49 ±0.007 | 0.46 ±0.008 | 0.45 ±0.01 | 0.42 ±0.01 | 0.38 ±0.01 |
| **Standard drug (100mg/kg)** | 0.32** ±0.009 | 0.52** ±0.007 | 0.47** ±0.007 | 0.45ns ±0.01 | 0.43** ±0.01 | 0.40** ±0.05 | 0.37ns ±0.01 |
| **Extract (250 mg/kg)** | 0.32** ±0.01 | 0.53* ±0.005 | 0.47** ±0.01 | 0.44** ±0.01 | 0.42** ±0.01 | 0.40** ±0.01 | 0.36ns ±0.02 |
| **Extract (500mg/kg)** | 0.33** ±0.01 | 0.52** ±0.001 | 0.48ns ±0.01 | 0.44** ±0.01 | 0.44ns ±0.01 | 0.39** ±0.02 | 0.35** ±0.02 |

Data are expressed as mean ±S.D. Significant at P<0.05*, P<0.01** and P<0.001*** and Non significant (ns), when compared to control n=6. *(P.A. - Plectranthus amboinicus)*

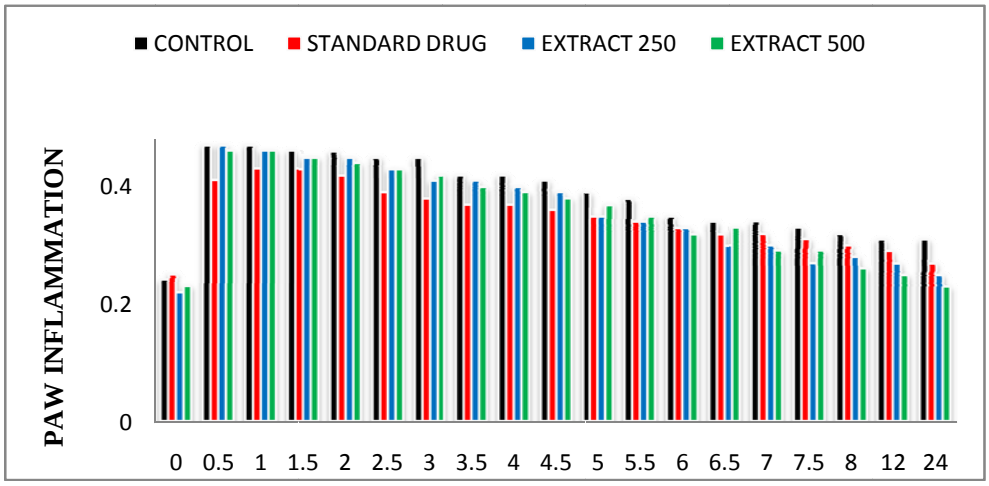
**Table 7: Effect of *P.A.* leaf extract on Egg-Albumin Induced oedema in mice**

| Treatment dose (mg/kg) | Time intervals(in hours) readings (in mm) | | | | | |
|---|---|---|---|---|---|---|
| | Initial 0th hour | 1st hour | 2nd hour | 3rd hour | 4th hour | 5th hour |
| Control (10ml/kg) | 0.36 ±0.005 | 0.48 ±0.008 | 0.45 ±0.01 | 0.42 ±0.01 | 0.41 ±0.006 | 0.40 ±0.004 |
| Standard drug (100mg/kg) | 0.31** ±0.005 | 0.45** ±0.005 | 0.42** ±0.008 | 0.40ns ±0.007 | 0.38ns ±0.008 | 0.36** ±0.01 |
| Extract (250 mg/kg) | 0.31** ±0.009 | 0.44** ±0.01 | 0.42** ±0.01 | 0.38** ±0.005 | 0.36** ±0.005 | 0.34 ±0.003 |
| Extract (500mg/kg) | 0.32** ±0.003 | 0.43** ±0.001 | 0.40** ±0.02 | 0.36** ±0.003 | 0.35** ±0.003 | 0.34** ±0.001 |

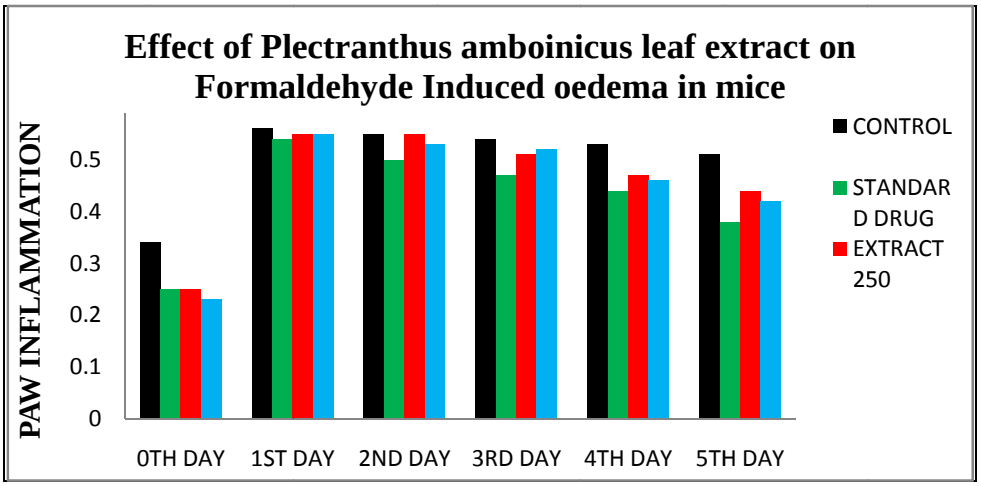
Data are expressed as mean ±S.D. Significant at P<0.05*, P<0.01** and P<0.001*** and Non significant (ns), when compared to control n=6. *(P.A. - Plectranthus amboinicus)*

**Table 8: GC – MS Analysis Obtained From The Essential Oil Obtained From The Fresh Leaves Of *Plectranthus Amboinicus***

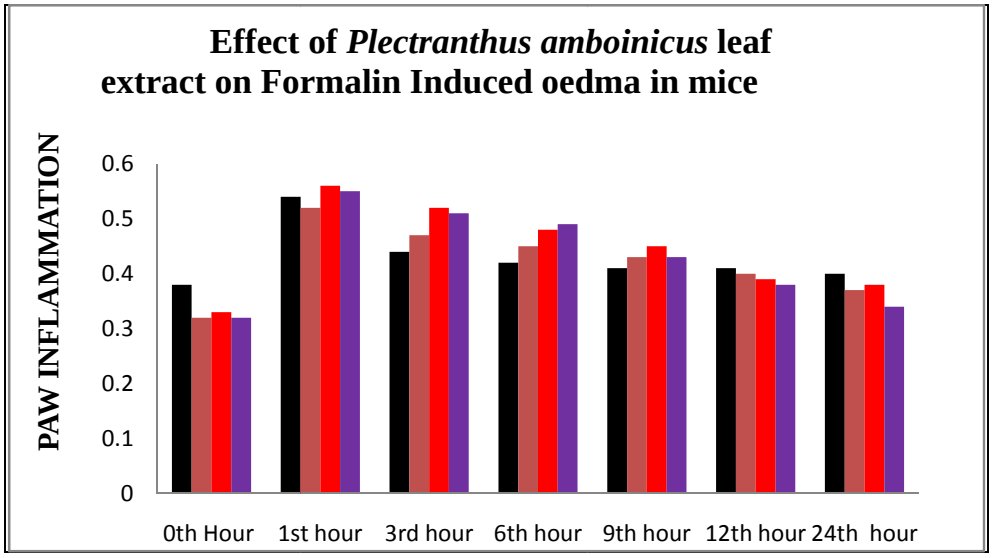
| S.no | Name of the plant | Compounds identified (in nos) | Major compounds |
|---|---|---|---|
| 1. | Plectranthus Amboinicus | 11 | Carvocrol -14%, Thymol – 18%, Cis –Caryophyllene, t-Caryophyllene, p-cymene -10% |



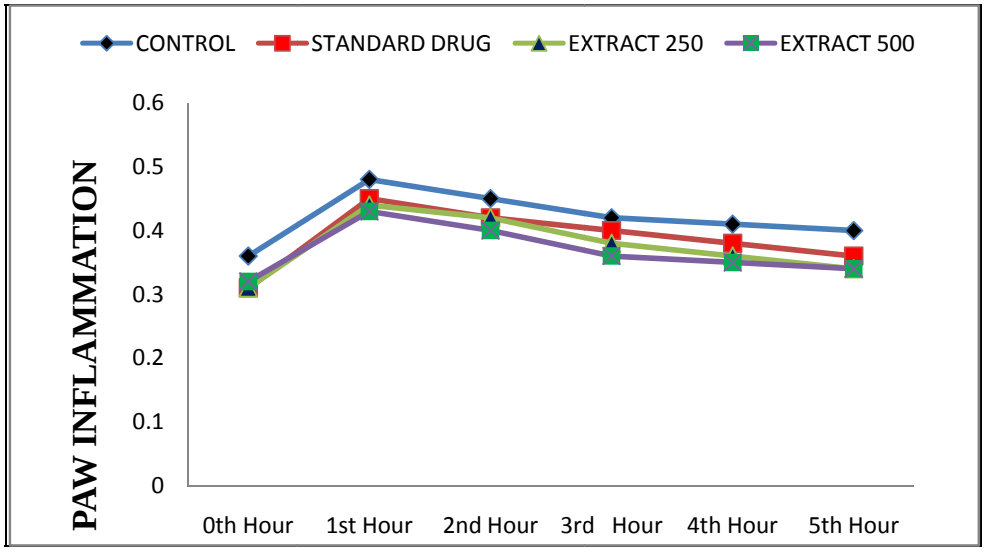

**Graph1: Effect of *P.A.* leaf extract on Carrageenan Induced oedema in mice**



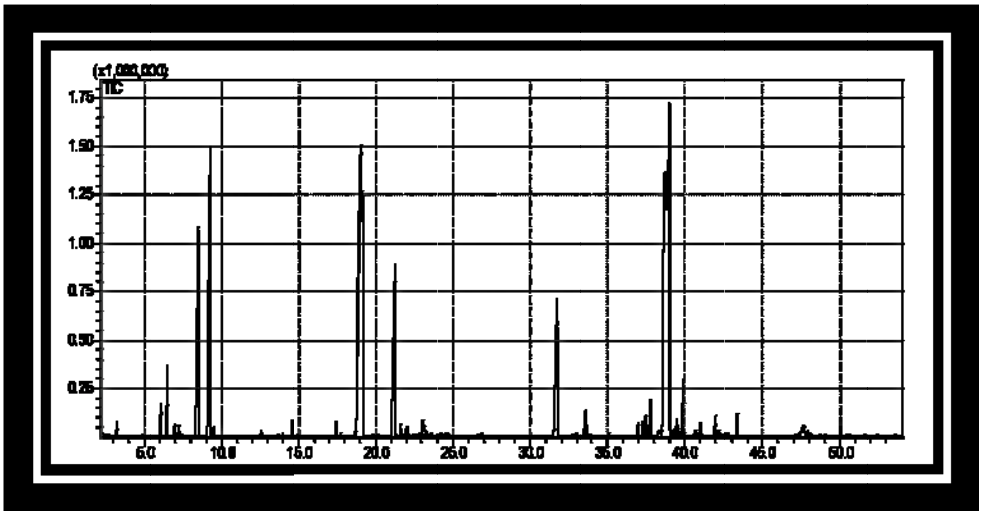

**Graph 2: Effect of *P.A* .leaf extract on Formaldehyde Induced oedema in mice**




**Graph3: Effect of *P.A.* leaf extract on Formalin Induced oedema in mice**




**Graph 4: Effect of *P.A.* leaf extract on Egg-Albumin Induced oedema in mice**




**Graph 5: GC-MS analysis of essential oil obtained from *P.A* Leaves**

GC profile of volatile oil of *P. amboinicus* dry leaf

**RESULT**

**Anti – fungal activity**

Table 1 shows the Minimal Fungicidal Concentration of *Plectranthus amboinicus* leaf extract which shows the MFC of different concentration of plant extract varying from 50-500µg/ml over different pathogenic fungal species. The result reveals that only 150, 250 and 500 µg/ml possess significant inhibitory activity over the fungal species and table 2 shows the Zone of inhibition of *Plectranthus amboinicus* leaf extract with a significant value (p<0.05-0.01) *Aspergillus.flavus* and *Aspergillus. niger* shows a less inhibition activity which shows a significant value of (P<0.05) when compared with the positive control the standard antifungal discs.

**Anti – Inflammatory activity**

**Carrageenan induced oedema in mice**

The results of the effect of methanolic extract of *Plectranthus amboinicus* on Carrageenan – induced oedema is shown in Table 3. The extract exerted a moderate anti-inflammatory effect at the highest dose 500mg/kg which was non-significant between 0.5- 2nd hour with a P value of (P<0.01) and it was non- significant at 3.5 and 6.5 hour interval and later 4 – 5.5 hours readings were significant with a P value of (P<0.05-0.01) and showed moderate to high significance of P value (P<0.05-0.01) from 7th hour to 24th hour post induction. But whereas, in 250mg/kg the extract showed a significant reduction in the paw volume where P value seems to be between (P<0.05 -0.01) from 1st hour to 24th hour and initially it seems to be non – significant from 0.5 to 2nd hour. However, the standard drug Diclofenac caused a significant reduction of oedema caused by Carragenan.

**Formaldehyde induced oedema in mice**

The effect of methanolic extract of *Plectranthus amboinicus* on formaldehyde induced oedema is shown in the Table 4. The extract exerted a strong anti-inflammatory effect at the highest dose and lowest dose (250-500mg/kg) which was moderate to highly significant with a P value of (P<0.05 -0.01) from the 1st day to 5th day of post induction. However, the standard drug Diclofenac, shows a

significant (P<0.01) reduction of oedema caused by formaldehyde when compared with control readings.

**Xylene induced oedema in mice**

Anti-inflammatory activity effect of leaf extract of *Plectranthus amboinicus* against xylene induced ear oedema in mice is shown in Table.5. The extract exerted a moderate anti-inflammatory activity which was only significant (P<0.05 – 0.01) with both lower and higher concentration of doses of plant extract when compared to that of the standard drug Diclofenac (100mg/kg).

**Formalin induced oedema in mice**

The result of the effect of methanolic extract of *Plectranthus amboinicus* on formalin induced oedema is shown in the Table 6. The extract treated animals showed a significant (P<0.05-0.01) dose related reduction of paw caused by formalin when compared with the standard drug and control. However, the anti-inflammatory effect of the extract was less than that of the standard drug Diclofenac.

**Egg albumin induced oedema in mice**

Table 7 shows the result of the effect of *Plectranthus amboinicus* on egg albumin induced oedema in mice. The extract exerted a considerable decrease in the paw volume when administered with 250 and 500mg/kg of extract which was only significant (P<0.05-0.01) when treated against oedema caused by egg albumin, comparable to that of the standard drug Diclofenac.

**DISCUSSION**

In this study pharmacological evaluation of antifungal and anti–inflammatory activities of methanolic extract of *Plectranthus amboinicus* was carried out using different experimental models. It is important to investigate scientifically plants that have been used in traditional medicines to determine potential sources of novel antimicrobial compounds [18]. Determination of Antifungal activity of *Plectranthus amboinicus* leaf extract at different concentration of 150,250, and 500µg/ml possess an inhibitory effect against the fungal species taken for the study. However our findings indicate that all the tested fungal species were susceptible and some are resistant to the standard anti fungal disc Fluconozole.

Carrageenan is the sulphated polysaccharide obtained from seaweed, which is widely used phylogistic agent which shows signs and symptoms of inflammation, that can be assessed as increase in paw thickness in mouse as a result of increased inflammation, edema and increased vascular permeation. Inflammation produced by Carrageenan is a triphasic response. In the first phase of inflammation, histamine , serotonin and other primary mediators are involved which cause the oedema and redness. In the second phase, different cytokines and kinnins get released in response to the inflammation and the secreted mediators at the localized site. In the third phase, the COX enzyme plays pivotal role leading to the production of prostaglandins which induces pain (17).

In the present study, *Plectranthus amboinicus* leaf extract showed inhibition of paw thickness at $3^{rd}$–$5^{th}$ hour and $7^{th}$-$24^{th}$ hours which probably suggested that *Plectranthus amboinicus* leaf extract inhibit the prostaglandin formation in the third phase of inflammation.

The extract was effective in formaldehyde and egg albumin oedema showing that it inhibits inflammation by blocking the release of histamine and 5-HT, two mediators that are released by egg albumin. This further point of the fact that the inhibition observed above in Carragenan induced oedema was not due to inhibition of histamine and 5-HT, but possibly kinnins or other mediators which are involved in this process . However Diclofenac, a cyclooxygenase inhibitor reduced significantly oedema produced by both formaldehyde and egg albumin.

The leaf extract exerted a significant (P<0.01) inhibition of ear oedema caused by xylene only at the highest dose of the extract (500mg/kg). This suggests the inhibition of phospholipase $A_2$ which is involved in the pathophysiology of inflammation due to xylene. However, Diclofenac a steroid anti-inflammatory agent produced significant reduction in the mean right ear weight of positive control mice indicating an inhibition of $PLA_2$. This action of the extract resembles that of Para-amino phenol group of the NSAIDS which possess anti-inflammatory effect particularly in peripheral tissue. The experimental evidence obtained in this study indicates that the extract reduced formalin induced paw oedema in mice. Similarly, formalin exhibits neurogenic and inflammatory pains indicate narcotic involvement.

These results pattern portrays a similarity in mode of action of paracetamol which has been reported to lack or possess a weak anti-inflammatory activity but has a strong wound healing property and acts as a sedative. Paracetamol has been reported to inhibit centrally the synthesis of prostaglandin in the brain by inhibiting COX – 3. However, it does not inhibit peripheral biosynthesis of prostaglandins and therefore lacks peripheral anti-inflammatory effect.

**CONCLUSION**

To conclude the study, the extract has demonstrated significant anti-fungal and moderate to high anti-inflammatory activity when compared with standard drug. These actions are exerted through central activity of the plant extract in the brain. These findings confirm its traditional medicinal use in the treatment of several inflammatory and painful conditions. The study reveals that both the antifungal and anti-inflammatory activity of the plant extract is dose dependent. Further study is required to find out the accurate compound responsible for the plant's medicinal value. We hope that our study emphasizes the accuracy and efficacy of traditional remedies, and that it inspires people to realize the importance of protecting natural resources for sustainable use, not in the least for its potent pharmaceuticals. This study also illustrates the strong dependence of certain people on traditional medicine and the creativity in which plants and their secondary metabolites can be utilized. Moreover comparative study of medicinal plants gives a vast idea about the plants nature and their medicinal value from its essence of traditional usage.

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