

# Evaluation of Anti-Inflammatory and Anti-Bacterial Activities of Different Solvent Extracts of *Ehretia laevis* Roxb.

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## Abstract:

### Aim:

To evaluate the anti-inflammatory and anti-bacterial activities of different solvent extracts of *Ehretia laevis* Roxb.

### Methods:

In this study we evaluate the anti-inflammatory and anti-bacterial activities of different solvent extracts of *Ehretia laevis* Roxb, for the anti-inflammatory activity inflammation is induced by Carrageenan induced rat paw edema method and anti-bacterial activity done by using Agar well diffusion method. For anti bacterial activity the microorganisms used were as follows, *Pseudomonas aeruginosa* NCIM 2036, *Staphylococcus aureus* ATCC BAA 1026, *Bacillus subtilis* ATCC 11774 and *Escherichia coli* ATCC 10536 and *Aspergillus niger*- NCIM 616. In anti-inflammatory activity The percentage inhibition of edema volume was calculated and in anti bacterial activity Diameter of the zone of inhibition was measured and the average diameter for each sample was calculated.

### Results:

The chloroform extract, methanolic and aqueous extract of *Ehretia laevis* show good anti-inflammatory activity by reducing paw volume at different doses. All extract has shown excellent antibacterial activity against gram negative and gram positive organism. When compared to chloroform, methanol and aqueous methanolic extract showed the high antibacterial activity on gram positive and gram negative bacteria.

### Conclusion:

The present study reveals the existence of anti-inflammatory and antimicrobial activities of different solvent extracts of *Ehretia laevis* Roxb. This investigation is limited and these results helpful for further investigation of *Ehretia laevis* Roxb plants to assess their chemical prospective in future research.

**Key words:** anti-inflammatory, antimicrobial, Carrageenan, diffusion, *Ehretia laevis* Roxb.

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## INTRODUCTION:

Pathogenic bacteria have always been considered as a major cause of morbidity and mortality in humans. Even though pharmaceutical companies have produced a number of new antibacterials in the last years, resistance to these drugs has increased and has now become a global concern [1]. The global emergence of multi-drug resistant (MDR) bacteria is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure [2]. Bacterial resistance to chemically unrelated antimicrobial agents is public health concern [3] and may be caused by over-expression of MDR efflux pumps [4]. In Gram-negative bacteria, the effect of the efflux pumps in combination with the reduced drug uptake (due to the presence of a double membrane barrier) is responsible for the high inherent and acquired antibiotic resistance often associated with this group of organisms [5]. Among Gram-negative bacteria, many of these MDR efflux pumps belong to the RND (resistance-nodulation-cell division) type family of tripartite efflux pumps. Due to the increase of resistance to antibiotics, there is a pressing need to develop

new and innovative antimicrobial agents. Among the potential sources of new agents, plants have long been investigated. Because, they contain many bioactive compounds that can be of interest in therapeutic. Because of their low toxicity, there is a long tradition of using dietary plants in the treatment of infectious disease in Cameroonian folk medicine. Consequently, we focused one of the objectives of our research group at investigating the antibacterial potentials of such plants against MDR phenotypes. There are several plants in which the analgesic and anti-inflammatory activity was reported earlier with the better curability and effectiveness. Here in this, work it is wished to find out a better source for the analgesic and anti-inflammatory activity with comparison to other medicinal plants.

The uses of traditional medicine wide spread medicine and plants still represent a large source of natural anti-oxidants that might serve as leads for the development of the novel drugs. Several anti-inflammatory, digestive, antinecrotic, neuroprotective and hepatoprotective drugs have recently been shown to have an antioxidant or radical scavenging

mechanism as part of their activity. The mechanism of inflammation injury is attributed, in part, to release of Reactive Oxygen species from activated neutrophil and macrophages. This over production leads to tissue injury by damaging the macromolecule and lipid peroxidation of membranes. In addition, ROS propagate inflammation by stimulating the release of the cytokines such as interleukin-I, tumor necrosis factor- $\alpha$ , and interferon- $\gamma$ , which stimulate recruitment of additional neutrophil and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation. Most clinically important medicine belongs to steroidal or non-steroidal anti-inflammatory chemical therapeutics for treatment of inflammation related diseases. Though these have potent activity and long term administration is required for treatments of chronic diseases. Furthermore, these drugs have various and severe adverse effects. Therefore, naturally originated agents with very little side effects are desirable to substitute chemical therapeutics. Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease predominantly affecting the joints and periarticular tissues. RA still remains a formidable disease, being capable of producing severe crippling deformities and functional disabilities. RA is classified as an inflammatory arthritis, the disease comprises of 3 basic inter-related processes like inflammation, synovial proliferation and joint tissue destruction. RA factor containing immune complexes found in the joints activate the pathological process. Tumour necrosis factor alpha (TNF-alpha) is the product of macrophages has been demonstrated to play an important role in the pathogenesis of RA. *A. malabarica* L is a shrubby, erect, densely tomentose or thickly woolly; stems slightly branched, obtusely quadrangular, clothed with soft woolly hairs. Leaves are very thick, oblong-lanceolate, acute, and pale above, white below, crenate-serrate, base rounded or shortly cuneate; petioles long, stout, softly woolly. In the present study, we investigated whether these plants have anti-arthritis, anti-platelet and anti-inflammatory activities [6].

#### MATERIALS AND METHODS:

##### Collection of plant material:

The plant *Ehretia laevis* was collected from in forest area of near Tirupati hills, Chittoor district, AP, India. The parts of the plant were separated, cleaned with sterile cotton and were shade dried. After the complete dry, plant parts were powdered separately and the powders were preserved in air tight container and were used when required. For the isolation of medicinal molecules and evaluation of the pharmacological activity of the plant, equal weight of the plant powder was mixed and the mixture was used as whole plant powder for the further work.

##### Extraction procedure:

The powdered material was weighed in a fixed quantity and was subjected to soxhelt extraction using Chloroform, Methanol and Water in successive mode respectively for 48h. The extraction was continued till colorless solvent appear in the thimble of soxhelt extractor. The solvent was

then recovered using distillation apparatus and the concentrated extract was further evaporated using a rotator vacuum evaporator to get dry powder separately for each of the solvent. The dried powder was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation of pharmacological activities[7].

##### Chemicals and reagents:

Nutrient agar medium for bacterial strains are used for studying the antimicrobial property. Amoxicillin drug was used as a standard for antibacterial activity.

##### Cultures used for antimicrobial activity:

The microorganisms used were as follows, *Pseudomonas aeruginosa* NCIM 2036, *Staphylococcus aureus* ATCC BAA 1026, *Bacillus subtilis* ATCC 11774 and *Escherichia coli* ATCC 10536 and *Aspergillus niger*- NCIM 616.

##### Preparation of plant extract:

Series of extraction methods were conducted with the whole plant *Ehretia laevis roxb* by chloroform methanol and water solvents using soxhlet extractor.

#### Methods

##### Anti-inflammatory activity of *Ehretia laevis* Roxb By Carrageenan induced rat paw edema method:

For determining the anti-inflammatory activity of the selected plant, plethysmograph method by measure the paw volume was followed. Male albino rats (weighing between 150 - 200 g) were divided into 5 groups (n=6 animals in each group). Group I served as control (vehicle treated) 10 ml/kg for seven days, group II served as positive control (Aspirin treated) 20 mg/kg for seven days, groups III, IV, V received 100, 200 and 400 mg/kg of different solvent *Ehretia laevis* Roxb for seven days. A mark was made on both the hind paws just below the tibio-torsal junction. After Thirty minutes of treatment, an inflammatory edema was induced in the left hind paw by injection of 0.1 ml of 1% of Carrageenan solution in the plantar tissue of the paw of all the animals. The right paw served as a reference. [8,9]The initial paw volume was measured plethysmographically within 30 seconds after injection. The relative increase in paw volume was measured in all groups at 30 minutes, 1, 2 and 3 hrs after Carrageenan injection. The percentage increase in the paw volume over the initial reading was calculated. This increase in the paw volume in-group II, III, IV and V were compared with group I. The percentage inhibition of edema volume was calculated by using the formula[10].

$$\text{Percentage of inhibition} = 100 (1 - V_t / V_c)$$

Where,

V t is increase in paw volume in treated groups

V c is increase in paw volume in control groups

##### Antibacterial Activity by Agar well diffusion method:

Each Petri dish containing nutrient agar medium was inoculated with one bacterial culture by spreading the suspension of the organism with a sterile glass rod with a bended tip. In each plate cups of 6mm diameter were made at equal distances using sterile cork borer. The extracts of plants were tested. All plates were kept in the refrigerator for 30 minutes to allow the diffusion of sample to the surrounding agar medium. The petri dishes were incubated at 37°C for 24 hrs. The wells were created using a stainless

steel sterilized cork borer under aseptic conditions. The chloroform, methanolic and aqueous solvent extracts of two plants were tested at different concentration. Amoxicilline was used as standard. The plates were incubated at room temperature for 48 h and zones of inhibition were measured. Diameter of the zone of inhibition was measured and the average diameter for each sample was calculated. The diameter obtained for the test samples were compared with standard[11,12].

#### RESULTS AND DISCUSSION:

The chloroform extract of *Ehretia laevis* Roxb at 100 mg/kg body weight per day when given orally as a suspension the paw volume were reduced by 1.24% whereas in case of 200 mg/kg body weight per day when given orally as a suspension the paw volume were reduced by 1.19% whereas in case of 400 mg/kg body weight per day shows 0.79% inhibition after 3h which indicate that effect of chloroform extract of *Ehretia laevis* Roxb is reflected in dose dependent manner. Results of the % inhibition of paw edema for chloroform extract were given in table 1.

The Methanolic extract of *Ehretia laevis* Roxb at 100 mg/kg body weight per day when given orally as a suspension the paw volume were reduced by 0.63% whereas in case of 200 mg/kg body weight per day when given orally as a suspension the paw volume were reduced by 0.42% whereas in case of 400 mg/kg body weight per day shows 0.32% inhibition after 3h which indicate that effect of Methanolic extract of *Ehretia laevis* Roxb is reflected in dose dependent manner. In this extract the % inhibition of Methanolic extract was found to similar to the standard. Results were given in table 2

The aqueous extract of *Ehretia laevis* Roxb at 100 mg/kg body weight per day when given orally as a suspension the paw volume were reduced by 0.96% whereas in case of 200 mg/kg body weight per day when given orally as a suspension the paw volume were reduced by 0.75% whereas in case of 400 mg/kg body weight per day shows 0.47% inhibition after 3h which indicate that effect of aqueous extract of *Ehretia laevis* Roxb is reflected in dose dependent manner. Results of the % inhibition of paw edema for chloroform extract were given in table 3. % inhibition activity was found to be less than the methanolic extract.

**Table :1 Anti-inflammatory Activity results of Chloroform Extract of *Ehretia laevis* Roxb**

Group	Dose	% inhibition of paw edema after carrageenan injection			
		30min	1Hour	2Hours	3Hours
Group I	Control 10ml/kg	1.42	1.51	1.59	1.62
Group II	Positive Control 20mg/kg	0.92	0.87	0.52	0.21
Group III	100mg/kg	1.40	1.35	1.31	1.24
Group IV	200mg/kg	1.34	1.26	1.21	1.19
Group V	400mg/kg	1.23	1.07	0.94	0.79

**Table :2 Anti-inflammatory Activity results of Methanolic Extract of *Ehretia laevis* Roxb**

Group	Dose	% inhibition of paw edema after carrageenan injection			
		30min	1Hour	2Hours	3Hours
Group I	Control 10ml/kg	1.42	1.51	1.59	1.62
Group II	Positive Control 20mg/kg	0.92	0.87	0.52	0.21
Group III	100mg/kg	1.26	1.07	0.85	0.63
Group IV	200mg/kg	1.12	0.91	0.75	0.42
Group V	400mg/kg	1.07	0.89	0.61	0.32

**Table :3 Anti-inflammatory Activity results of Aqueous Extract of *Ehretia laevis* Roxb**

Group	Dose	% inhibition of paw edema after carrageenan injection			
		30min	1Hour	2Hours	3Hours
Group I	Control 10ml/kg	1.42	1.51	1.59	1.62
Group II	Positive Control 20mg/kg	0.92	0.87	0.52	0.21
Group III	100mg/kg	1.35	1.29	1.14	0.96
Group IV	200mg/kg	1.21	1.13	0.89	0.75
Group V	400mg/kg	1.17	0.95	0.61	0.47

#### Antimicrobial activity:

The antibacterial activity of the chloroform, methanolic and aqueous solvents leaf antibacterial activity of the ethyl chloroform, methanolic and aqueous solvents leaf extracts of the plant was depends on their phyto chemical composition. The diameters of the inhibition zones were measured in millimetre. The preliminary phytochemical components of the plants were studied various bioactive compounds were reported The demonstration of antimicrobial bacterial activity against both gram positive and gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds. All extract has shown excellent antibacterial activity against gram negative and gram positive organism. When compared to chloroform, methanaol and aqueous methanolic extract showed the high antibacterial activity on gram positive and gram negative bacteria and *aqueous extracts* shows the high antibacterial activity on gram negative than gram positive. Results of zone of inhibition were presented in table 4. This will be of immense advantage in fighting the menace of antibiotic refractive pathogens that are so prevalent in recent times. However, actual antibacterial ingredients need to be extracted and identified also its tolerable levels in the human body as well as any toxic effects on human and animal tissues are investigated accordingly.

**Table 4: Antimicrobial activity of *Ehretia laevis* roxb**

Name of the microorganism	Type of extract	Size of zones (in mm) observed for different concentrations (in µg/ml)					
		1000 (µg/ml)	500 (µg/ml)	250 (µg/ml)	100 (µg/ml)	50 (µg/ml)	10 (µg/ml)
<i>E.coli</i>	Methanol	24.43	22.25	16.8	7.1	4.1	3.8
	chloroform	20.7	19.7	14.7	5.5	4.0	-
	Aqueous	21.7	17.4	10.6	5.8	-	-
<i>Staph. aureus</i>	Methanol	21.6	19.3	17.1	5.2	-	-
	chloroform	22.9	20.8	15.7	5.4	-	-
	Aqueous	22.7	21.4	17.1	7.1	4.3	-
<i>P. aeruginosa</i>	Methanol	25.1	22.8	16.7	5.3	4.5	-
	chloroform	24.1	24.9	18.4	7.9	4.8	-
	Aqueous	23.7	22.3	19.4	8.7	5.6	-
<i>B.subtilis</i>	Methanol	25.4	21.2	16.2	4.7	-	-
	Chloroform	20.9	21.7	19.7	9.2	6.7	-
	Aqueous	22.7	19.8	15.3	6.6	5.7	-

## Discussion

Nature has been a source of medicinal agents for thousands of years. Various medicinal plants have been used for years in daily life to treat disease all over the world. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. The most important of these biologically active constituents of plants are alkaloids, flavonoids, tannins and phenolic compounds. There are many herbs, which are predominantly used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders.

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5 year period. The present review aims to compile data generated through the research activity using modern scientific approaches and innovative scientific tools in last 5 year period i.e. 1994-1998. Medicinal plants have assumed greater importance in the recent days, due to the tremendous potential they offer in formulating new drugs which afflict humankind against many diseases. [13]

Hence evaluation of pharmacological activities likes anti-inflammatory and antibacterial activities of chloroform, Methanolic and aqueous extracts of *Ehretia laevis* Roxb whole plant was studied. *Ehretia laevis* Roxb is an Indian medicinal plant belongs to Boraginaceae family and having ayurvedic importance. In some parts of India, a decoction of the fresh root is used for the treatment of syphilis, whereas a decoction of the stem bark is used for the treatment of diphtheria in some other parts. Hence anti-diabetic, anti-inflammatory and antioxidant activity of *Ehretia laevis* Roxb was studied.

Shxhlet extraction method was followed for the isolation of phyto-constituents from the whole plant *E laevis* Roxb using chloroform, Methanolic and Water as solvent in series extraction method. The crude extract obtained was weighed and it was found that for 1kg of the plant contain 26.85gr of compounds extract with chloroform, 168.19gr in methanol and 86.74gr are extracted using water solvent.

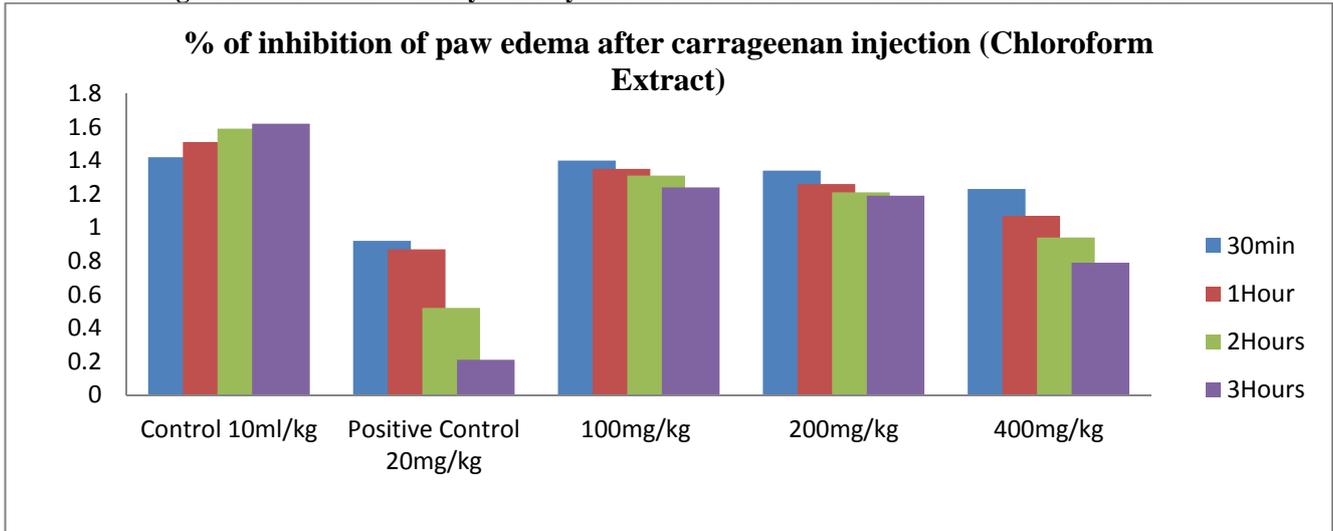
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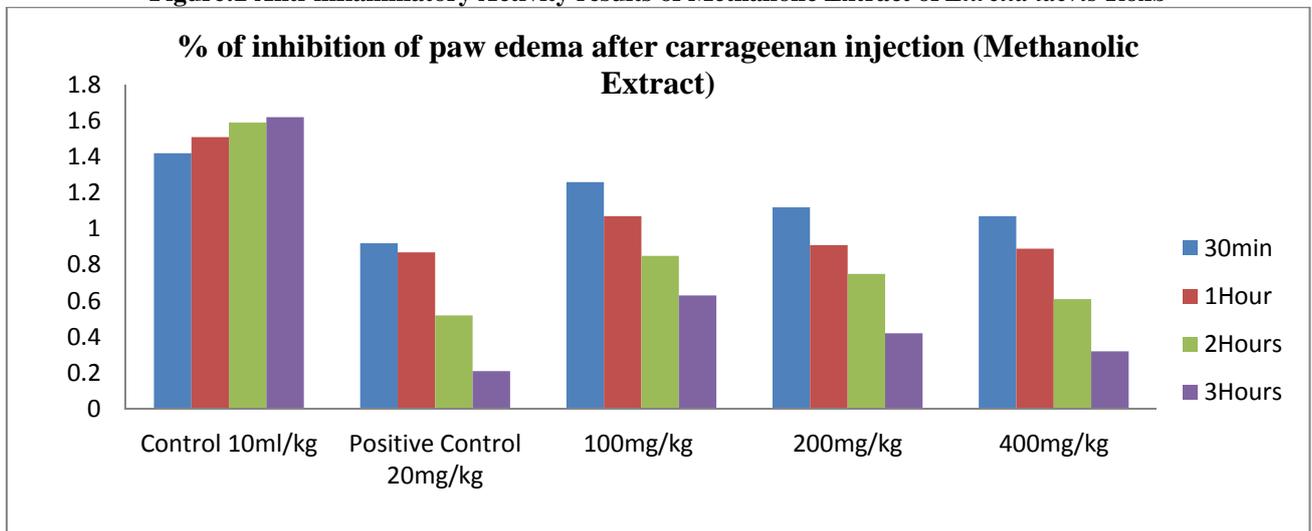
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The preliminary phytochemical components of the plants were studied various bioactive compounds were reported The demonstration of antimicrobial bacterial activity against both gram positive and gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds. All extract has shown excellent antibacterial activity against gram negative and gram positive organism. When compared to chloroform, methanol and aqueous methanolic extract showed the high antibacterial activity on gram positive and gram negative bacteria and *aqueous extracts* shows the high antibacterial activity on gram negative than gram positive.

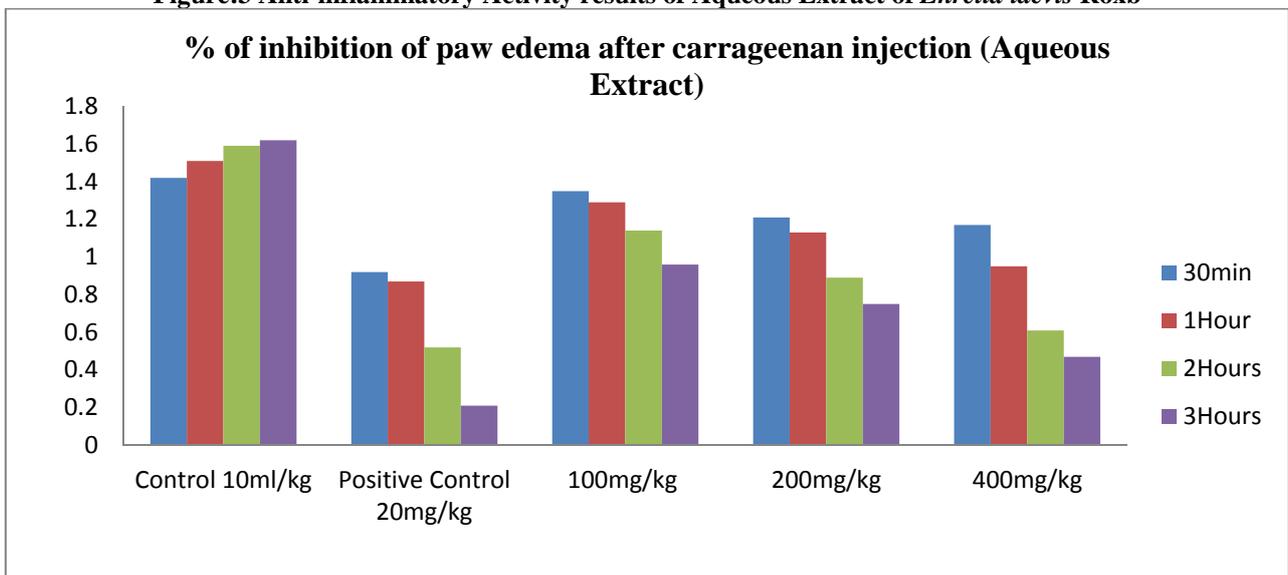
**Figure:1 Anti-inflammatory Activity results of Chloroform Extract of *Ehretia laevis* Roxb**

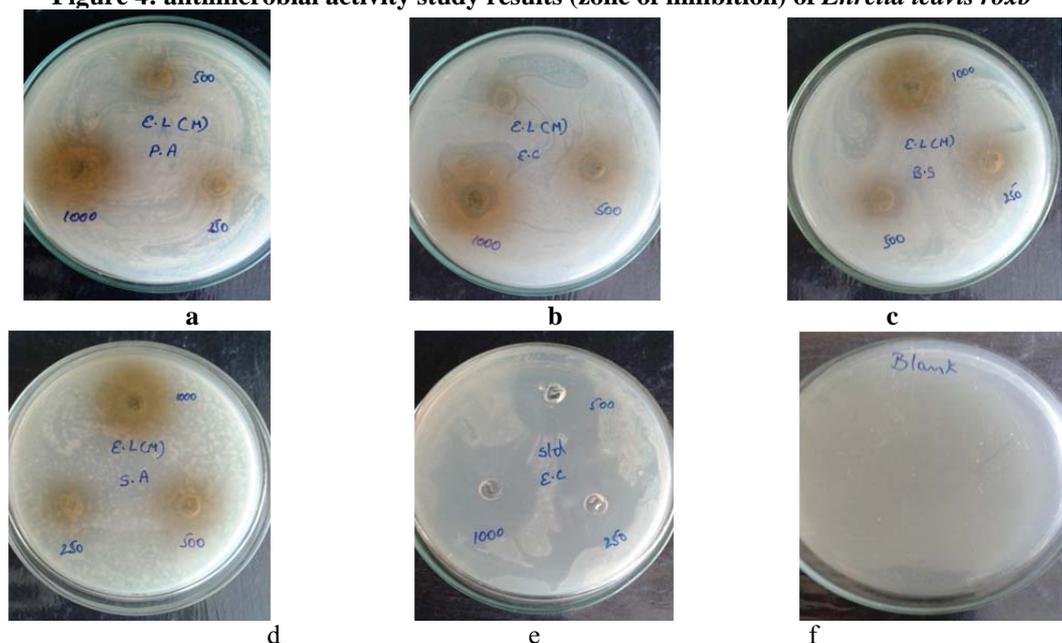


**Figure:2 Anti-inflammatory Activity results of Methanolic Extract of *Ehretia laevis* Roxb**



**Figure:3 Anti-inflammatory Activity results of Aqueous Extract of *Ehretia laevis* Roxb**



**Figure 4: antimicrobial activity study results (zone of inhibition) of *Ehretia leavis roxb***

- a. *ehretia leavis roxb* methanolic extract (*Pseudomonas aeruginosa*)  
 b. *ehretia leavis roxb* methanolic extract (*Escherichia coli*)  
 c. *ehretia leavis roxb* methanolic extract (*Bacillus subtilis*)  
 d. *ehretia leavis roxb* methanolic extract (*Staphylococcus aureus*)  
 e. standard drug (*Escherichia coli*)  
 f. Blank

#### CONCLUSION

The preliminary phytochemical analysis shows the presence of many constituents which would have played a role in the pharmacological activities studied. The present study provided results to justify the traditional claim of herbs for anti-inflammatory activity. The reported activities of the extracts make the whole plant more valuable in treating not only anti diabetes but also the associated secondary disorders. The plant can also be further explored for its activity against wide spectrum of microbes and can be developed into a powerful antibiotic. The screening of antimicrobial activity performed with different extracts of *Ehretia leavis roxb* proved that the plant having antimicrobial compounds. The current work will provide new reference data for the drug development and possesses the ability to inhibit pathogenic bacteria. Further studies should be done on fractionation and identification of bioactive constituents which are responsible for antibacterial activity.

#### ACKNOWLEDGMENT:

The authors are thankful to Principal and management of Narasaraopeta Institute of Pharmaceutical Sciences, for the providing the facilities for the conducting this work.

#### REFERENCES:

- Adwan G, Mhanna M: Synergistic effects of plant extracts and antibiotics on *staphylococcus aureus* strains isolated from clinical specimens. *Journal of Scientific Research*. 2008, 3: 134-139
- Hancock EW: Mechanisms of action of newer antibiotics for Gram-positive pathogens. *Lancet Infect Dis*. 2005, 5: 209-218. 10.1016/S1473-3099(05)70051-7.
- Sharma R, Sharma CL, Kapoor B: Antibacterial resistance: current problems and possible solutions. *Indian J Med Sci*. 2005, 59: 120-129. 10.4103/0019-5359.15091.
- Li XZ, Nikaido H: Efflux-mediated drug resistance in bacteria. *Drugs*. 2004, 64: 159-204. 10.2165/00003495-200464020-00004.
- Lomovskaya O, Bostian KA: Practical applications and feasibility of efflux pump inhibitors in the clinic—a vision for applied use. *Biochem Pharmacol*. 2006, 71: 910-918. 10.1016/j.bcp.2005.12.008.
- Basavaraj Chivde V, Karnakumar Biradar V, Rajabhau S, Shiramane, Kamshetty Manoj V. In vitro antioxidant activity studies of the flowers of *Tagetes erecta* L. (Compositae). *International Journal of Pharma And Bio Sciences*, 2(3), 2011, 223-229.
- Kirtikar KR, Basu BD. *Indian Medicinal Plants*. Edn 2, Lalit Mohan Basu, Allahabad, India, 1961, pp. 314-315.
- Jing Zhen, Yue Guo, Tom Villani, Steve Carr, Thomas Brendler, Davis R. Mumbengegwi, Ah-Ng Tony Kong, James E. Simon, and Qingli Wu1, *Phytochemical Analysis and Anti-Inflammatory Activity of the Extracts of the African Medicinal Plant Ximenia caffra*, *Journal of Analytical Methods in Chemistry*, Volume 2015 (2015), Article ID 948262, 9 pages.
- C. O Okoli., P. A . Akah and Ezugworie, U, anti-inflammatory activity of extracts of root bark of *securidaca longipedunculata* fres (polygalaceae), *Afr. J. Trad. CAM* (2005) 2 (3): 54 – 63.
- M.J. Abada, P. Bermejoa, E. Carretero, C. Martínez-Acitoresa, B. Noguera, A. Villar, *Antiinflammatory activity of some medicinal plant extracts from Venezuela*, *Journal of Ethnopharmacology*, Volume 55, Issue 1, December 1996, Pages 63–68
- CLSI, *Performance Standards for Antimicrobial Disk Susceptibility Tests*, Approved Standard, 7th ed., CLSI document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, 2012.
- CLSI, *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts*, Approved Guideline. CLSI document M44-A. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA, 2004.
- Anjaneya S Ravipati1, Lin Zhang, Sundar Rao Koyyalamudi, Sang Chul Jeong1, Narsimha Reddy, John Bartlett, Paul T Smith, Kirubakaran Shanmugam, Gerald Münch, Ming Jie Wu, Manavalan Satyanarayanan and Balaram Vyset, *Antioxidant and anti-inflammatory activities of selected Chinese medicinal plants and their relation with antioxidant content*, *BMC Complementary and Alternative Medicine* 2012, 12:173.