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Anti-inflammatory and Antibacterial Activity of Aerial Part of *Justicia gendarussa*

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Abstract

Justicia gendarussa is important plant for medicinal purposes and utilized from ancient periods for the treatment of pain, inflammation, higher sugar level as antibacterial and anti-inflammatory activity. The present investigation has evaluate the anti-inflammatory activity of *Justicia gendarussa* in freund's complete adjuvant induced arthritic rats. The anti-inflammatory and antimicrobial activity of Justicia gendarussa leaves was examined by rat's paw edema. The active constituents' justicidine and phyllamyricin are responsible of anti-inflammatory activity

Keywords: *Justicia gendarussa*, Antiinflammatory activity, Carrageen, Antibacterial Activity

Introduction

Medicinally valued plants has role in modern medicine and therapeutics. Modern medicine has limitations as these may produce drug induced toxicity to major organs such as liver, kidney, brain and heart etc. Researchers consider all the time drug efficacy with patient safety is utmost parameter while developing or manufacturing the drugs or dosage forms. Ancient medicine system, Ayurveda, unani, siddha & naturopathy has been used from the down to earth for treatment of disease. Many medicinal plants not yet identified for the curing diseases, lack of literature. In the global scenario traditional natural medicines obtained from plants has been used in continents and usage of herbal is goes on increasing continuously. Applications of natural medicines further continued especially in countries like in South Asia. Indians blessed with god's gift especially in area of medicine system i.e., Ayurved.

Objectives

- To Identification of phytochemical composition of roots and aerial part of Justicia gendarussa
- To evaluate in vivo Antiinfflamatory and Antimicrobial activity

Plan of Work

- Collection, Drying and grinding of leaves.
- Authentication of the plant.
- Determination of physiochemical parameter.

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- Extraction of leaves with different solvents.
- Procurement of animals and their diet.
- Evaluation of extracts in animals for their Antiifflammatory and Antibacterial activity
- Interpretation of result by statistical analysis.

Material and methods

Materials

List of Chemicals & Solvents

Acetone: Merck Specialties (MS) Pvt. Ltd., Mumbai
Chloral hydrate: MS Pvt. Ltd., Mumbai
Chloroform: Rankem, New Delhi
Ethyl acetate: Rankem, New Delhi
Ethyl alcohol: SD fine chem. Ltd., Mumbai
Sodium carbonate: Qualigens Fine Chemicals, Mumbai
Sodium cromoglycate: Ipca Ltd., Goa. India
Sodium hydroxide: MS Pvt. Ltd., Mumbai
Potassium hydroxide: MS Pvt. Ltd., Mumbai
Sulphuric acid: Qualigens Fine Chemicals, Mumbai
Histamine diphosphate: Sigma Aldrich, USA.
Hydrogen peroxide: MS Pvt. Ltd., Mumbai
Methanol (HPLC Grade): MS Pvt. Ltd., Mumbai
Millon's reagent: CDH Pvt. Ltd., New Delhi

List of Equipments

Ashless filter paper: Qualigens Fine chemicals, Mumbai Autoclave: Hi-con, New Delhi
B.O.D. Incubator: Scope Burette stand: Dolphin Burette stand: Dolphin Butter paper: ASGI® Camera:Sony
Centrifuge: REMI
Centrifuge machine: Remi, Mumbai Clavengers apparatus: Borosil Colorimeter: Equiptronics
Compound microscope: Getner Kywo, Ambala Condenser: ASGI®
Crucible: Borosil
Deep Freezer: Remi, India Desiccator: Tarson
Digital Balance (1mg Sensitivity): Contech
Digital Elevated plus Maze: Dolphin Drying Oven: CINTEX
Electric Water Bath: Hi-con, New Delhi
Haemocytometer: Marinfeld, Germeny
Heating mantle: ASGI®
Metabolic cages: Dolphin
Micro pipette: Biosystem
Micropipette: Vertex Pvt. Ltd.
Soxhlet apparatus: Borosil Soxhlet extractor: ASGI® Spatula: ASGI® Stirrer/glass rod: ASGI®
Ultrafast Liquid Chromatography: Schimadzu UV Cabinet: Biotechnics
UV Chamber: Hi-con, New Delhi
UV –Vis spectrophotometer: Schimadzu Vernier calipers: Coslab, Mumbai Watch glass: ASGI®
Petri Plates: ASGI®
Water bath: Hi-con, New Delhi
Whatmann filter paper: Manipore microproducts, Mumbai
Hot plate method (Eddy and Leimbach, 1953)
Pleythysmometer (Almemo, 2290-4)

Plant Description

Name - *Justicia gendarussa*
Family - Acanthaceae
Genus - Justicia
Synonyms - Gendarussa vulgaris
Common name - Ganda rusa
Chemical constituents: - 2-amino benzyl Alcohol, Urosolic acid, beta sitosterol lupeol, and stimosterol
Distribution - It is native to china and it is widely grow throughout in India.
Uses - It is used as a herbal medicine, roots and leaves contain bitter alkaloid (justicine), It is used as inflammation, pain, fever, bronchitis, vaginal discharges, chronic rheumatism.

Figure 1: Aerial part of *Justicia gendarussa*

Biological name : ***Justticia gendarusa***
Family : *Acanthaeceae*
Synonyms:
Hindi name : Nilie narrgandi
Kannada : aduthodagida, karalakigidde
Bengal : jagatmadan
Tamil : karunochhi, vadaikkuti
Telugu : adasaramu, gandhrasamu
Marathi : tevv, bakass
Sanskrit : bhuttakeshi, gandharasae

The Justicia gendarussa is found in china in folklore medicines and native to china but it is found in India thoughout the country.

The plant aerial part and leaves are utilized as antibacterial, Anthelmintic, anti-inflammatory, anti-arthritic activity, analgesic, female contraceptives and in constipation.

Plant material collection and authentication

The *Justicia gendarussa* leaves were collected from Nagpur District, Maharashtra, India

In the month of September 2023 The plant was identified and authenticated by R M Acharya, Post graduate teaching Department of Botany,Wardha, Rashtrasant Tukadoji Maharaj, Nagpur University, Nagpur. A voucher specimen No.9409 was deposited with the post graduate Teaching Department of Botany, Wardha, Nagpur University, Nagpur.

Extraction of *Justicia Gendarussa* leaves

The shade dried and powered leaves of *justicia gendarussa* leaves, were subjected to successive extraction in a soxhlet apparatus with petroleum ether (60-80º), chloroform, methanol and finally macerated with water so

as to get respective extract. All extracts were individual filtered, through Whatsman filter paper ± 42 and evaporated to dryness at 50ºC in oven. The extracts were then stored in desiccators till further use.[72]

Extraction process

Soxhlet apparatus was used for continuous extraction of the powdered crud drug. The material was packed in the apparatus and allowed to get extracted with hot solvent that continuously percolates from top to bottom. Condensed fresh solvent percolates every time through the powder and is the major advantage with this technique. The powder was extracted using solvents petroleum ether (60-80º), chloroform and methanol respectively for 24 hrs. The ratio of powder to solvent was 10:100.

Petroleum ether (60-80º) extraction

Dried powder was charged in soxhlet apparatus and first extracted with petroleum ether to remove fatty material. After the extraction process, solvent was distilled off and the extract was dried at 50ºC. Dried extract was stored in desiccators till further use.

Chloroform extraction

Mark obtained from petroleum ether extraction was air dried and extracted with Chloroform. After the extraction process, solvent was distilled off and the extract was dried at 50ºC. Dried extract was stored in desiccators till further use.

Methanol extraction

Mark obtained from Chloroform extraction was air dried and extracted with Methanol. After the extraction process, solvent was distilled off and the extract was dried at 50ºC. Dried extract was stored in desiccators till further use.

Water extraction

Mark obtained from Methanol extraction was air dried and extracted with macerated at room temperature. The extract was dried at 50ºC. Dried extract was stored in desiccators till further use.

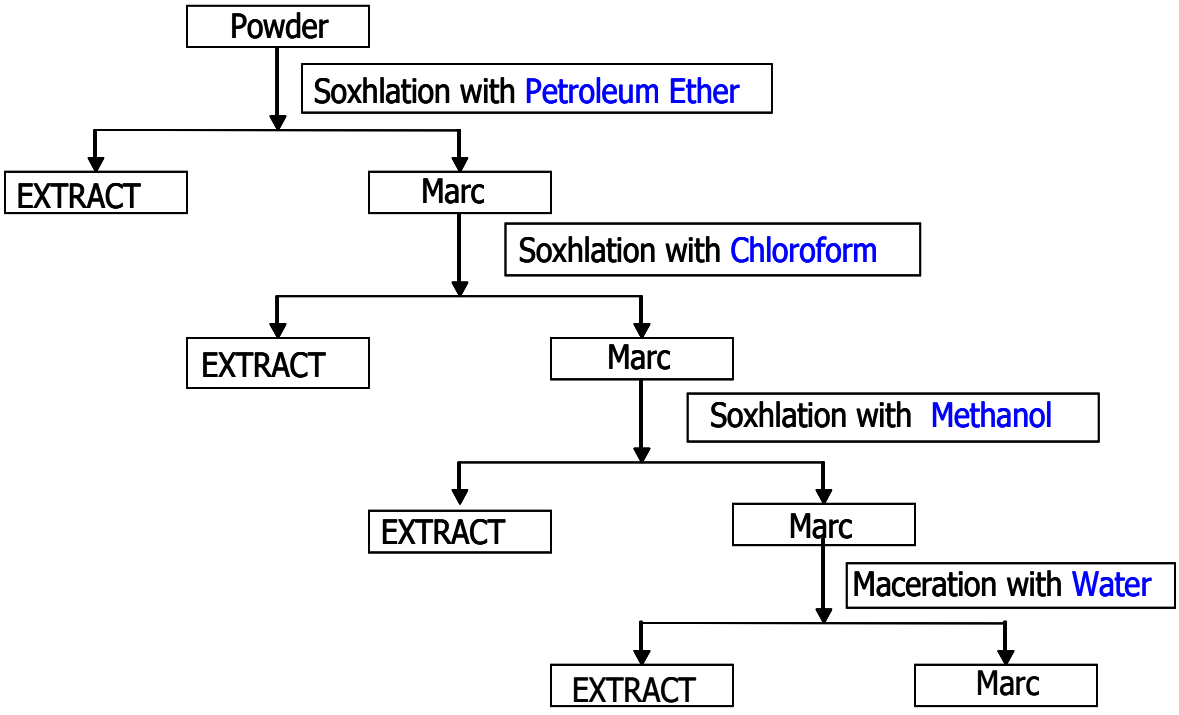

Figure 2: Flow chart showing extraction process of *Justicia gendarussa* leaves

Table 1: Extractive valves of *Justicia gendarussa* leaves extracts.

Sr. No.	Solvent	Extraction Process	% Yield
1	Petroleum ether(60-80º) (PEE)	Soxhlation	8.2%
2	Chloroform (CLE)	Soxhlation	6.5%
3	Methanol (MEE)	Soxhlation	12.3%
4	Water (WAT)	Maceration	18%

Procurement of Experiment animals:

The animals bred in the animal house of VIVA Institute of Pharmacy,Virar (E) Maharashtra, were procured for the experiment. The animal were house in polypropylene cages at a temperature of 25 ± 2ºC with relative humidity of 40-60% and 12:12 hour light dark cycle. Animal were fed with a balance diet and water ad *libitum* during the complete experiment period. All animal experiment were approved by the Institutional Animal Ethical Committee VIVA Institute of Pharmacy Animal Facility, Virar (E.) 2001/PO/Re/S/18/CCSEA, 21st February 2018..) of VIVA Institute of Pharmacy, Virar (E), Maharashtra . These all animals are kept in animal house.

Aim and objective of the test

The aim of the test is to obtain using a minimum no of animals, sufficient information on the acute toxicity after single administration, by oral rout in the rat, of a test substance, for its classification.

Test substance administered to a group of experiment animals by oral route at one defined dose (5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg) according to the available information on test substance. The liquid preparation was given less than 1 ml/100 gm body wt. of animals Animal were observed after one hour at least, after administration to detect signs of toxicity.

Animals used

Male albino rats, weighing 150-200 gm were used. They were housed in the standard environmental condition and fed with diet with water. The animals were housed in 37cm ×23cm ×16cm polypropylene cages with maximum of 6 animals per cage. The cages were placed in limited access premises of animal house with controlled temperature and humidity. The artificial lighting ensured a sequence of 12 hours light and 12 hours dark.

Test procedure

Carrageenan- induced rat paw edema

Animals were fasted for 24 h before the experiment with free access to water approximate 0.1 ml of a 1% suspension of carragenan in saline was prepared 1 h before each experiment and was injected into the planter side of right hind paw of rat. The rat were divided into six groups (n=6). Group 1 served as control and received normal saline and the group 2,3 and 4 were treatedorally with aq extract, pet ether and ethanol extract 250-500 mg/kg b.w., respectively. Group 5 received the standard drug aceclofenac (10 mg kg^{-1}) the aq extract, pet ether and ethanol extract was administrated 1 h prior to injection of 0.1 ml of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw sub planter of each rat. The paw volume was measured initially and then at 1, 2, and 3 h after the carrageenan injection by using plethysmometer. The anti-inflammatory effect was calculated by the following equation:

Anti-inflammatory activity (%) = (1-Vt/Vc) ×100

Where, Vt represents the paw volume in drug treated animals and Vc represent the paw volume of control group animals.

Analgesic activity

Hot plate method

The analgesic activity of different extract was assessed using as described by hot plate method of Eddy and Leimbach (1953). The evaluated parameters were the latency time for paw licking and jumping responses on exposure to the hot plate surface which is kept at 55±1ºC. The animals were kept in the hotplate until it lifted one of its hind paws. For this method, the rats were divided into 6 groups of 6 animals each. Group I served as control (5% gum acacia, 1 ML 100 g^{-1}), group II, III and IV received aqueous extract, petroleum ether extract and ethanol extract at a dose of 300 and 1500 mg kg^{-1} orally. Group V received Asprin at a dose of 25 mg kg^{-1}. All the treatments were given 30 min before the thermal stimulus and the response was determined at 60, 120 and 180 min.

Table 2: Effect of different extract of *Justicia gendarussa burm.* leaves on carrageenan induced rat paw edema for '0' hr

Treatments	Dose	Paw volume (ml)
Control	10 mL kg^{-1}	0.252±0.020
Aceclofenac	10 mg kg^{-1}	0.220±0.021
WTE	1500 mg kg^{-1}	0.246±0.024
PEE	300 mg kg^{-1}	0.243±0.022
ETE	300 mg kg^{-1}	0.236s±0.018

N=6. P Value is not significant. Data were analyzed by one way ANOVA followed by Dunnett test

Testing Various portion range from 1000 to 32.5 g / ml using two overlap sequential weakening strategy alongside dissimilar bacterial strains was considered for in vitro activity for isolated fractions. According to the findings, F1 exhibited base antagonistic fixation of 250 to 500 g/ml of the stock against each bacterial strain. The positive control's results, which ranged from 1.8625 to 3.625 g / ml, were nearly identical. The same findings also revealed a obstruction zone using the cup plate strategy. In contrast to microscopic organisms, this medication demonstrated better movement alongside, leading us to select S. typi for our subsequent enemy bacterial review. Using two crease sequential weakening strategies and a variety of bacterial strains, the effects of Enicostemma littorale ranged from 1000 to 32.5 mg/ml. F2's findings revealed a base antagonistic focus of 250 to 500 g/ml stock against each bacterial strain. The results were nearly identical due to a positive MIC of 1.8625 to 3.625 g/ml. The cup plate technique was also found to cause zone obstruction in the comparative findings. S. Typi was chosen as our next enemy bacterial reexamination because medication demonstrated a healthier battle against other microbes. The results showed that F3 produced a base antagonistic concentration of 250 to 500 mg/ml. F4 demonstrated base antagonistic focus at concentrations ranging from 250 mg/ml to 500 mg/ml against each bacterial strain. The results were nearly identical, ranging from 1.8625 to 3.625 g/ml for the MIC. Our findings demonstrated that each division took action, but F4 produced more antibacterial movement than other groups. medications should have significant areas of strength in order for them to function at low doses. In the context of a transdermal prescription delivery system. Spread plate technique required the transfer of 20 milliliters of supplement agar media for each culture, which was transferred onto petri dishes with the help of a miniature pipette and a clean twisted glass pole. They were kept for the mandatory Methodology for Antibacterial Activity Testing of Drugs because they either suppress or inhibit the growth of microorganisms, which are typically broken down by microbial strategy. Cup plate strategy agar well dispersion technique was used in the testing.

Using an agar-well dissemination method and 0.1 milliliters of short-term culture, the antibacterial action concentrates were able to hatch for necessity. Cups made Petri plates using sterile fitting drill (compulsory) each concentrate was added each well point bacterial plates were incubated mandatory. Six bores were drilled into each test compound, each of which determined the zone of hindrance width mean. Antibacterial development directed by assessment zone of impediment around each well plate using zone peruser. Mean

width obligatory mm was recorded for the estimated restraint zones. Gentamycin hostile to contamination w used as required control.

Result and Discussion

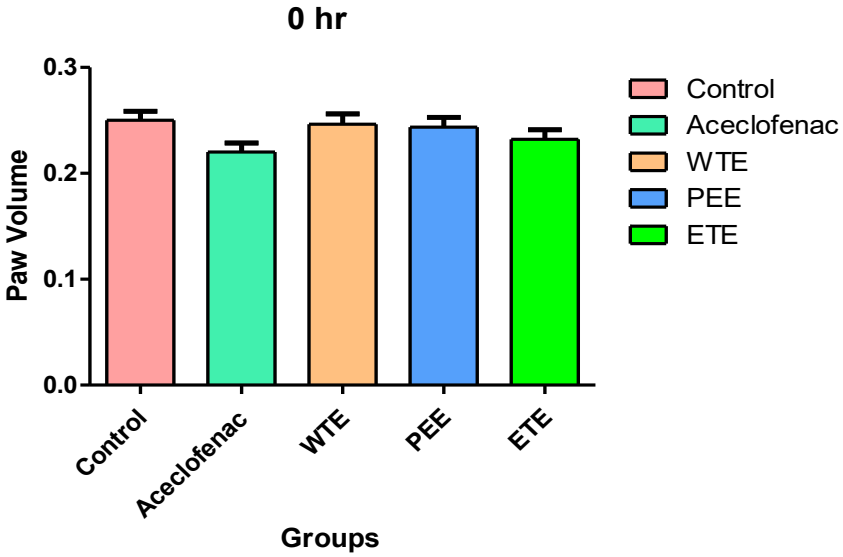

Figure 3: Graph showing change in the paw volume in '0'hr

Table 3: Effect of different extract of *Justicia gendarussa burm.* leaves on carrageenan induced rat paw edema for '1' hr

Treatments	Dose	Paw volume (ml)
Control	10 mL kg^{-1}	0.273±0.027
Aceclofenac	10 mg kg^{-1}	0.238±0.016
WTE	1500 mg kg^{-1}	0.247±0.023
PEE	300 mg kg^{-1}	0.250±0.021
ETE	300 mg kg^{-1}	0.230s±0.027

N=6. P Value is not significant. Data were analyzed by one way ANOVA followed by Dunnett test

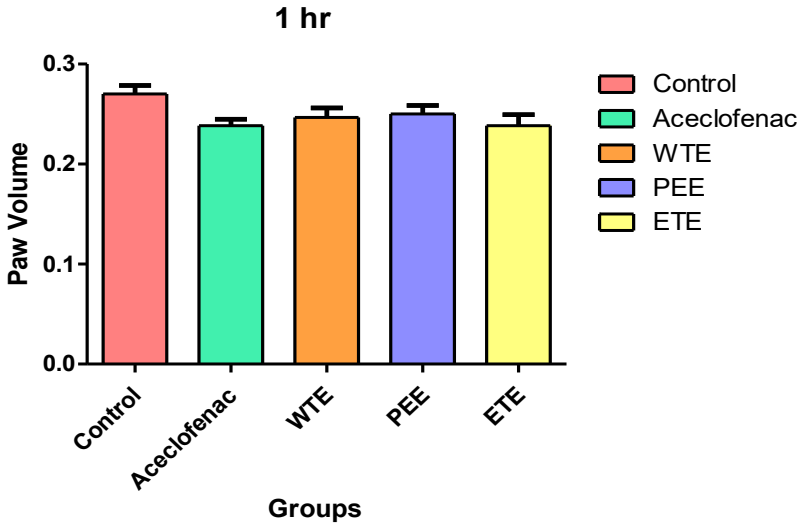

Figure 4: Graph showing change in the paw volume in '1' hr

Table 4: Effect of different extract of *Justicia gendarussa burm.* leaves on carrageenan induced rat paw edema for '2' hr

Treatments	Dose	Paw volume (ml)
Control	10 mL kg^{-1}	0.440±0.021
Aceclofenac	10 mg kg^{-1}	0.260±0.022*
WTE	1500 mg kg^{-1}	0.275±0.024*
PEE	300 mg kg^{-1}	0.240±0.022*
ETE	300 mg kg^{-1}	0.250±0.023*

N=6. *p<0.001 vs. control. Data were analyzed by one way ANOVA followed by Dunnett test

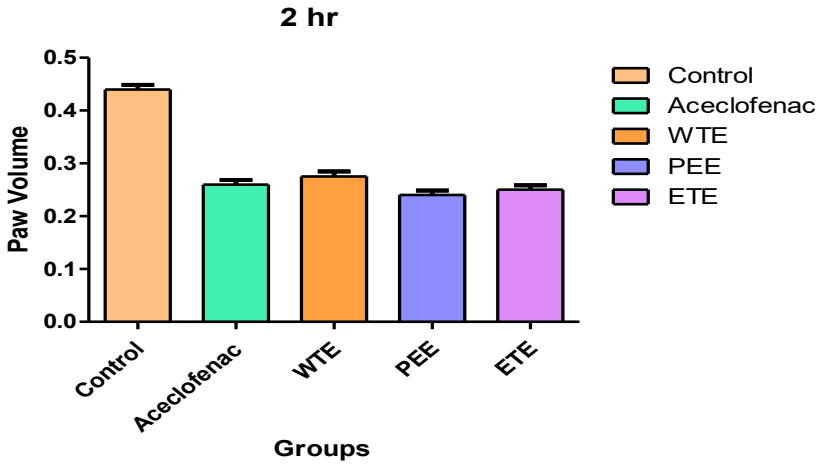

Figure 5: Graph showing change in the paw volume in '2' hr

Table 5: Effect of different extract of *Justicia gendarussa burm.* leaves on carrageenan induced rat paw edema for '3' hr

Treatments	Dose	Paw volume (ml)
Control	10 mL kg^{-1}	0.460±0.021
Aceclofenac	10 mg kg^{-1}	0.260±0.022*(47%)
WTE	1500 mg kg^{-1}	0.280±0.043*(39%)
PEE	300 mg kg^{-1}	0.290±0.024*(37%)
ETE	300 mg kg^{-1}	0.258±0.021*(44%)

N=6. *p<0.001 vs. control. Data were analyzed by one way ANOVA followed by Dunnett test

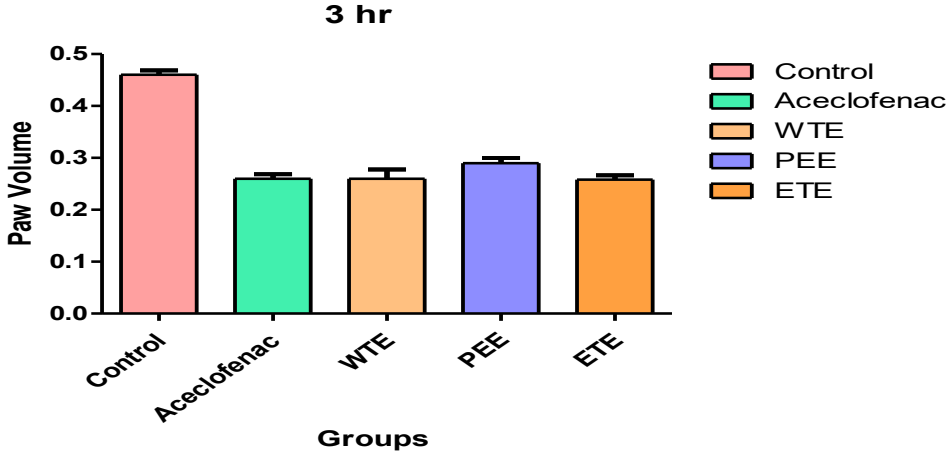

Figure 6: Graph showing change in the paw volume in '3' hr

Table 6: Effect of different extract of *Justicia gendarussa burm.* leaves on thermic stimulus induced (Hot plate) pain in rats for '0' hr

Treatment	Dose	Reaction time (sec)
Control	10 mL kg^{-1}	2.320±0.037
Asprin	25 mg kg^{-1}	2.570±0.183
WTE	1500 mg kg^{-1}	2.370±0.0284
PEE	300 mg kg^{-1}	2.420±0.0037
ETE	300 mg kg^{-1}	2.480±0.047

N=6. P Value is not significant. Data were analyzed by one way ANOVA followed by Dunnett test

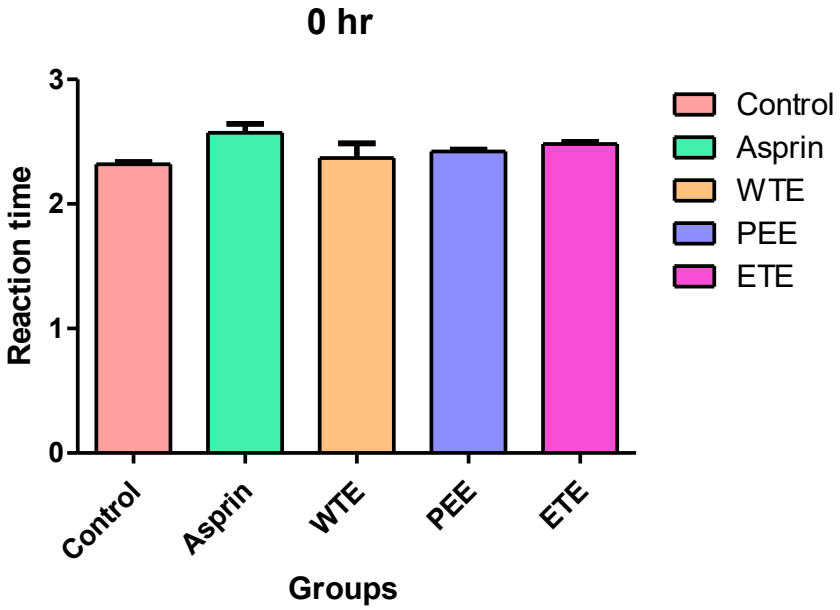

Figure 7: Graph showing change in reaction time in '0' hr

Table No. 7: Effect of different extract of *Justicia gendarussa burm.* leaves on thermic stimulus induced (Hot plate) pain in rats for '1' hr

Treatment	Dose	Reaction time (sec)
Control	10 mL kg^{-1}	2.252±0.078
Asprin	25 mg kg^{-1}	9.058±0.601*
WTE	1500 mg kg^{-1}	3.677±0.647s*
PEE	300 mg kg^{-1}	3.818±0.029*
ETE	300 mg kg^{-1}	6.925±0.729*

N=6. *p<0.001 vs. control. Data were analyzed by one way ANOVA followed by Dunnett test

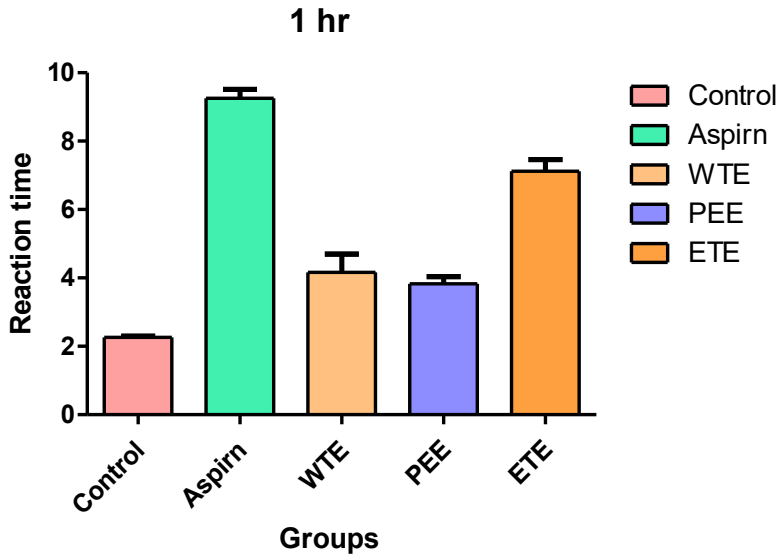

Figure 8: Graph showing change in reaction time in '1' hr

Table 8: Effect of different extract of *Justicia gendarussa burm.* leaves on thermic stimulus induced (Hot plate) pain in rats for '2' hr

Treatment	Dose	Reaction time (sec)
Control	10 mL kg^{-1}	2.445±0.360
Asprin	25 mg kg^{-1}	9.912±0.900*
WTE	1500 mg kg^{-1}	4.677±1.992*
PTE	300 mg kg^{-1}	5.158±1.634*
ETE	300 mg kg^{-1}	7.835±0.728*

N=6. *p<0.001 vs. control. Data were analyzed by one way ANOVA followed by Dunnett test

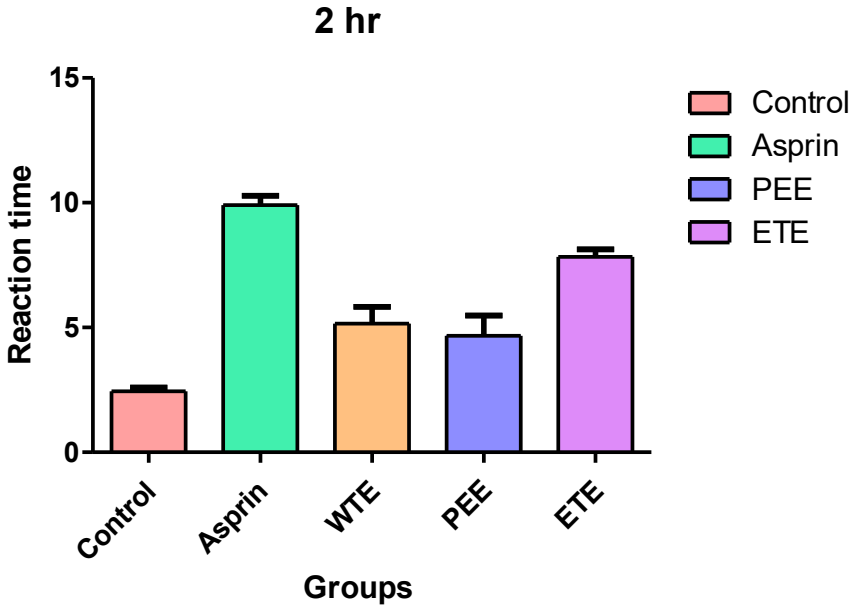

Figure 9: Graph showing change in reaction time in '2' hr

Table 9: Effect of different extract of *Justicia gendarussa burm.* leaves on thermic stimulus induced (Hot plate) pain in rats for '3' hr

Treatment	Dose	Reaction time (sec)
Control	10 mL kg^{-1}	2.312±0.023
Asprin	25 mg kg^{-1}	8.790±0.021*
WTE	1500 mg kg^{-1}	4.517±0.240*
PEE	300 mg kg^{-1}	4.853±0.132*
ETE	300 mg kg^{-1}	6.487±0.252*

N=6. *p<0.001 vs. control. Data were analyzed by one way ANOVA followed by Dunnett test

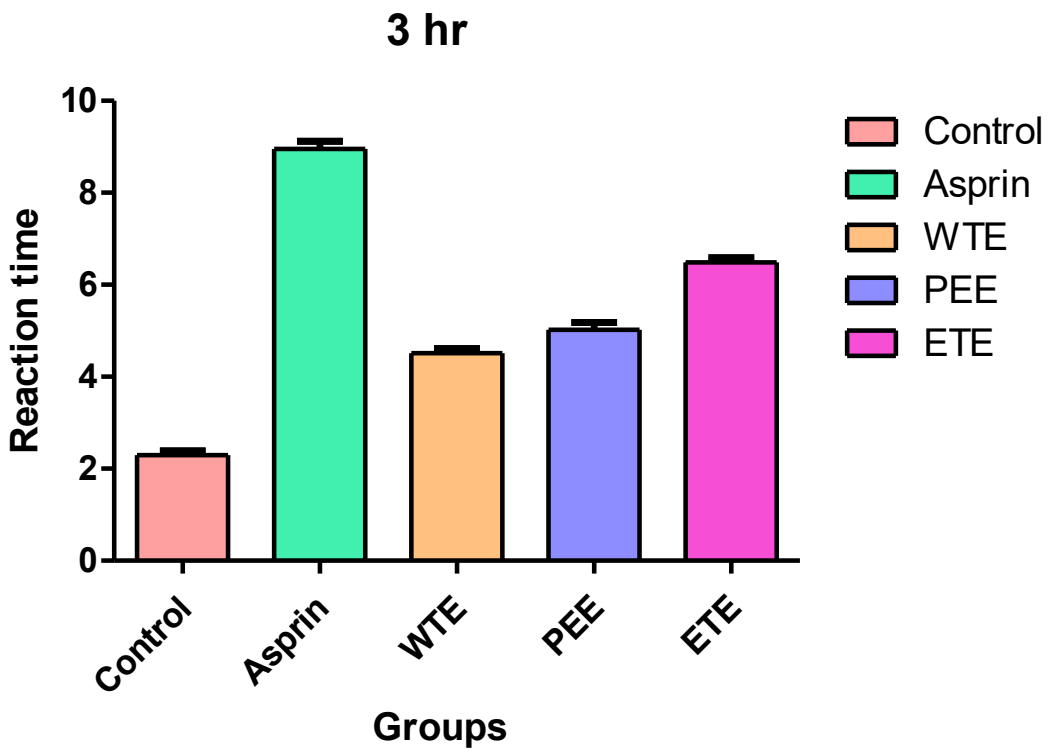

Figure 10: Graph showing change in reaction time in '3' hr

Table.: 10: Effect of fractions on selected bacteria strains (Two fold serial dilutions)

S. No.	Organisms	F1 MIC Values µg/ml	F2 MIC Values µg/ml	F3MIC Values µg/ml	F4 MIC Values µg/ml	Standard drug MIC Values µg/ml
1.	*S.typi*	500	250	250	500	3.625
2.	*S. enteritidis*	500	250	250	250	3.625
3.	*E.coli*	500	500	500	500	3.625
4.	*S. aureus*	500	500	250	250	1.8625
5.	*K. pneumoniae*	250	500	500	500	1.8625

Standard drug – Gentamycin

Table 11: Effect of fractions on selected bacterial strains (Cup Plate method)

Organisms	Concentration	Zone of inhibition(cm)								Standard drug Zone of inhibition values (cm)	
		F1		F2		F3		F4			
		W1	W2	W1	W2	W1	W 2	W1	Well 2	W1	W 2
S.typi	1000 µg/ml	1.2	1.4	1.6	1.1	1.1	1.3	1.7	1.2	1.3	1.6
	2000 µg/ml	2.1	2.4	2.9	2.1	2.1	2.4	3.1	2.1		
S. enteritidis	1000 µg/ml	1.3	1.4	1.7	1.2	1.3	1.42.	1.6	1.2	1.2	1.7
	2000 µg/ml	2.3	2.4	3.2	2.3	2.3	4	3.1	2.2		
E.coli	1000 µg/ml	1.2	1.1	1.9	1.1	1.1	1.2	1.9	1.1	1.8	1.9
	2000 µg/ml	2.7	2.1	4.0	2.6	2.7	2.1	4.0	2.5		
S. aureus	1000 µg/ml	1.5	1	1.7	1.5	1.6	1	1.7	1.5	1.8	1.7
	2000 µg/ml	2.7	2.3	3.6	2.7	2.7	2.3	3.6	2.8		
pneumoniae	1000 µg/ml	1.1	1.5	1.8	1.1	1.2	1.6	1.8	1.1	1.5	1.6
	2000 µg/ml	2.5	2.7	2.9	2.5	2.5	2.7	2.9	2.6		

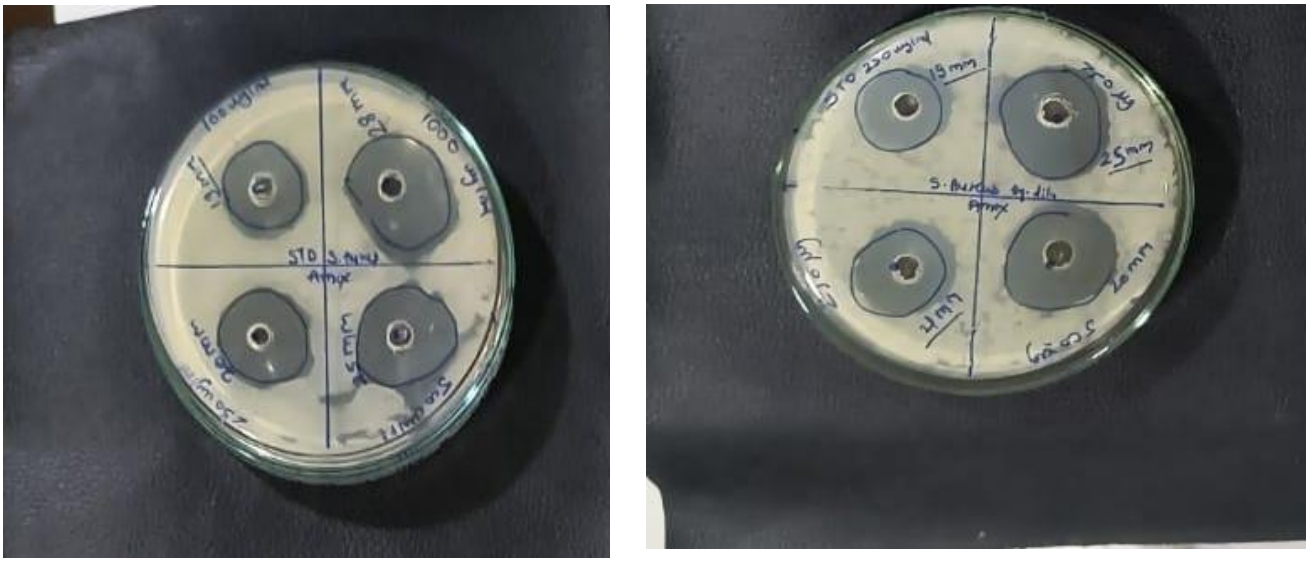

Figure 11: The plate showing antimicrobial activity of standard used for S. Aurius Gentamycin at different concentrations

Conclusion

The pharmacological activities were performed on animals like albino wistar rat. The anti-inflammatory activity was examined on rat in the groups of control, standard and test with 6 rats in each groups. The standard medicament was taken diclofenac for inflammation. The injection of carrageenan was administered in each rat's paw for induction of oedema or swelling. The diameter was measured with the vernier caliper and dosage of plant *Justica gendarusa* and standard medicament diclofenac was administered to different groups of animals. The dosages were given in interval of 1, 2, and 3 hours and the decrease in swelling is measured with screw gause. The reduction was found to be 45 pecent and compared to standard medicament 48 percent. The probability of results were $p<0.001$ for interval of 3 hours that is significant.

For analgesic activity of plant Justica gendarusa was carried out with hot plate click methods. The extracts of plant leaves with aerial part and standard analgesic medicaments were injected to the wister rat animals. The effect was noted in 1 hour intervals three times. The tail of rat was not removed on hot plate means extract are effective as analgesic as with standard medicaments. The comparison was found to 52 percent for extracts and 55 percent for standard medicament. MIC assurance The MIC of the concentrates was controlled by individually weakening each fixation (0.0-36, required mcg/ml). Comparable volume concentrates in the midst of supplement stock were mixed in the test tube. the explicit requirement of normalized inoculums containing the necessary additional cylinder. The cylinders brooded forcefully for the required 24 hours.

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