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Evaluation of *in-vitro* antibacterial activity and anti-inflammatory activity for different extracts of *Rauvolfia tetraphylla* L. root bark

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ABSTRACT

Objective: To assess the *in-vitro* antibacterial activity and anti-inflammatory activity of orally administered different extracts (Hydro-alcoholic, methanolic, ethyl acetate and hexane) of *Rauwolfia tetraphylla* (*R. tetraphylla*) root bark in Carrageenan induced acute inflammation in rats. **Methods:** *In-vitro* antibacterial activity was evaluated for extracts against four Gram positive and four Gram negative bacteria by using cylinder plate assay. Hydro-alcoholic extract (70% v/v ethanol) at 200, 400 and 800 mg/kg doses and methanolic, ethyl acetate and hexane extracts at doses 100, 200 and 400 mg/kg were tested for anti-inflammatory activity in Carrageenan induced rat paw oedema model and paw thickness was measured every one hour up to 6 hrs. **Results:** All extracts of *R. tetraphylla* root bark showed good zone of inhibition against tested bacterial strains. In Carrageenan induced inflammation model, hydro-alcoholic and methanolic extract of *R. tetraphylla* root bark at three different doses produced significant ($P<0.001$) reduction when compared to vehicle treated control group and hexane, ethyl acetate extracts. **Conclusions:** In the present study extracts of *R. tetraphylla* root bark shows good *in-vitro* antibacterial activity and *in-vivo* anti-inflammatory activity in rats.

1. Introduction

Natural products from plants may become a new source for development of drugs[1]. Although hundreds of plant species have been tested for pharmacological properties, the vast majority of have not been adequately evaluated[2,3]. Plants with their vast arrays of secondary metabolites form a reservoir of low molecular weight organic compounds that is largely untapped as a source of pharmaceuticals. Plants are used medicinally in different countries and are a source of many potent and powerful drugs[4]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds[5,6]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries[7]. In the recent years, Screening of medicinal plants for their biological activities and phytochemicals are important for finding potential new compounds for therapeutic uses.

Rauwolfia tetraphylla (*R. tetraphylla*) is a small tree shrub that will reach 6 feet (~2 meters) in height. Leaves are whorled, medium to dark green in color and occur in groups of 4 unequally-sized leaves at each node. The roots yield the drug deserpidine, which is an antihypertensive and tranquilizer.

In present study we have extracted dried root bark of *R. tetraphylla* in hexane, ethyl acetate, hydro-alcoholic (ethanol 70% v/v) and methanol. These extracts were checked for its antibacterial and anti inflammatory activities. The extracts were found to have a potent antibacterial and anti inflammatory effects.

2. Materials and methods

2.1. Preparation of extracts from R. tetraphylla root bark

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The roots of *R. tetraphylla* were collected from Araku, Visakhapatnam Dist, Andhra Pradesh, during the month of December, 2009. The authentication of the above plant was done by Dr. P. Prayaga Murthy, Department of Botany, Andhra University, Visakhapatnam. Shade dried root bark of *R. tetraphylla* was powdered and separately extracted in a Soxhlet apparatus for 6 hrs successively with hydro-alcoholic (ethanol 70%v/v), methanol, ethyl acetate and hexane were concentrated to dryness under vacuum.

2.2. Drug and chemicals

Indomethacin, sodium carboxy methyl cellulose (Na CMC) and Carrageenan were purchased from Sigma chemicals, USA. All other chemicals used were of analytical grade.

2.3. Evaluation of in-vitro antibacterial activity

The cylinder plate assay of drug potency is based on measurement of the diameter of zone of inhibition of microbial growth surrounding cylinders (cups), containing various dilutions of test compounds. A sterile borer was used to prepare four cups of 6 mm diameter in the agar medium spread with the micro-organisms and 0.1 mL of inoculums. These cups were spread on the agar plate by spread plate technique. Accurately measured (0.05 mL) solution of each concentration and reference standards were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8℃ for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37℃ for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity.

2.3.1. Test organisms

The microorganisms used for the experiments were procured from MTCC, IMTECH, Chandighar. Gram-positive organisms included *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus pumilis*. And Gram-negative organisms included *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Streptomyces marienensis*.

2.3.2. Culture media

For Anti bacterial activity of selected algae Muller-Hinton Agar media (Solid and Broth) was used. For maintaining the bacterial species Nutrient both was used.

2.4. Animals

Adult Wistar rats (National Institute of Nutrition, Hyderabad, India) of either sex weighing 200-250 g were used in the studies. The animals were maintained under standard laboratory conditions at an ambient temperature of (23-2)℃ having (50-5)% relative humidity with 12-h light and dark cycle. The use and care of the animals in the experimental protocol has been approved by the local Institutional Animal Ethics Committee (Regd. No. 516/01/A/CPCSEA) following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.4.1. Acute inflammation model: carrageenan induced rat paw oedema

Fourteen groups of rats were treated orally with 1% sodium CMC, 5 mg/kg Indomethacin and 200 mg/kg, 400 mg/kg and 800 mg/kg of different extracts of *R. tetraphylla* root bark respectively. Sixty minutes later, an injection of 1% carrageenan in normal saline was made into the subplantar region of the right hind paw of each rat in each group.

Before induction of oedema, the dorsiventral thickness of both the paws of each was measured using Zeitlin's apparatus[8] which consists of a graduated micrometer, combined with a constant loaded lever system to magnify the small changes in the paw thickness during the course of experiment. The measurements were taken at 1 hour intervals after induction of oedema for up to 6 hrs. Oedema was monitored as the percentage increase in paw thickness in the Carrageenan injected paw. To assess the oedema in control paw (right) saline was injected subcutaneously.

The percent inhibition of paw thickness is calculated using the formula:

Percentage inhibition = $100[1-(Y_t / Y_c)]$

Y_t = Average increase in paw thickness in groups tested with test compounds.

Y_c= Average increase in paw thickness in control.

2.4.2. Statistical analysis

Data of paw thickness was analyzed by using One-Way ANOVA followed by *post hoc* test Dunnett's test using Graph pad Prism-5 software. The results are expressed as Mean± SEM. $P<0.05$ was considered as significant.

3. Results

3.1. Evaluation of antibacterial activity

The hexane extract of *R. tetraphylla* root bark had not showed activity against *Streptococcus pneumonia*, *Enterobacter aerogens* and *Streptomyces marienensis* at a dose of 50 μg/cup. Ethyl acetate, methanol and hydro-alcoholic extracts (70% v/v ethanol) showed good zone of inhibition against tested bacterial strains at a dose of 150 μg/cup compared to hexane extract. The results were showed in Table 1.

3.2. Carrageenan induced rat paw oedema

The hydro alcoholic (200, 400 and 800 mg/kg) and methanolic extract of *R. tetraphylla* root bark at three different doses produced significant ($P<0.001$) reduction at 100, 200 and 400 mg/kg when compared to drug vehicle treated control group. The ethyl acetate and hexane extracts of *R. tetraphylla* root bark tested, at the doses 100, 200 and 400 mg/kg exhibited significant ($P<0.001$) activity, where as the dose 400 mg/kg exhibited a highly significant ($P<0.001$) effect when compared to drug vehicle treated control group. The results

Table 1
Zone of inhibition of *R. tetraphylla* root bark extracts against gram positive and gram negative bacteria (diameter in mm).

Extract of *R. tetraphylla* root bark	Dose(μg/cup)	gram (+)ve				gram (−)ve			
		S.p	*B.c*	*B.p*	*S.a*	*E.c*	*E.a*	*P.a*	*S.m*
Hexane	50	−	7	8	10	8	−	7	−
	100	8	10	12	12	11	10	10	8
	150	10	12	15	15	14	12	12	11
Ethyl acetate	50	9	10	11	11	10	9	10	8
	100	12	14	14	14	13	13	13	11
	150	16	17	16	18	17	17	15	15
Methanolic	50	9	9	10	9	10	11	8	10
	100	14	12	14	12	14	15	11	13
	150	18	16	17	16	17	18	15	17
Hydro-alcoholic (Ethanol 70%)	50	08	11	10	10	11	8	10	9
	100	13	14	13	14	13	12	13	13
	150	17	18	17	18	15	17	17	17
Standard Chloramphenicol	30	18	15	16	14	18	16	16	17
DMSO		−	−	−	−	−	−	−	−

B.s=Streptococcus pneumoniae; B.c=Bacillus cereus; B.p=Bacillus pumilis; S.a=Staphylococcus aureaus; E.c=Escherichia coli; E.a=Enterobacter aerogens; P.a=Pseudomonas aeruginosa; S.m=Streptomyces marienensis; −=No activity.
#Values are the average of triplicate; includes the cup diameter (6 mm).

Table 2
Effect of *R. tetraphylla* root bark extracts on Carrageenan induced rat paw edema (mean ± SEM).

Chemicals and Extract of *R. tetraphylla* root bark	Group	Dose	Mean increase in paw thickness at 6 h (mm)	Percent inhibition at 6 h (%)
1% Na CMC	Group I	1 mL	6.10±0.11	−
Indomethacin	GroupII	5 mg/kg	60.57±1.17*	68.32±1.47*
Hydro-alcoholic (70% ethanol)	Group III	200 mg/kg	20.13±1.30*	25.60±1.13*
	Group IV	400 mg/kg	32.25±2.30*	37.26±1.25*
	Group V	800 mg/kg	41.35±1.70*	46.91±1.03*
Methanol	Group VI	100 mg/kg	38.15±1.50*	42.68±0.80*
	Group VII	200 mg/kg	44.22±2.20*	47.49±1.15*
	Group VIII	400 mg/kg	47.15±1.64*	53.90±1.77*
Ethyl acetate	Group IX	100 mg/kg	20.14±1.20*	23.52±1.89*
	Group X	200 mg/kg	30.22±1.42*	35.21±1.17*
	Group XI	400 mg/kg	35.15±2.30*	39.57±0.61*
Hexane	Group XII	100 mg/kg	17.25±0.60*	21.67±0.82*
	Group XIII	200 mg/kg	20.32±1.30*	24.13±1.52*
	Group XIV	400 mg/kg	27.43±2.12*	32.99±1.74*

*Significance at $P < 0.001$. All groups were compared with control group.

were showed in Table 2, Figure 1-4.

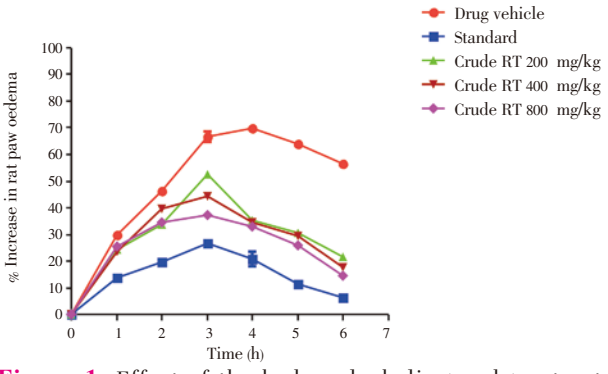

Figure 1. Effect of the hydro-alcoholic (crude) extracts of *R. tetraphylla* root bark at 200, 400 and 800 mg/kg along with indomethacin (2.5×10^{-5} moles/kg body weight).

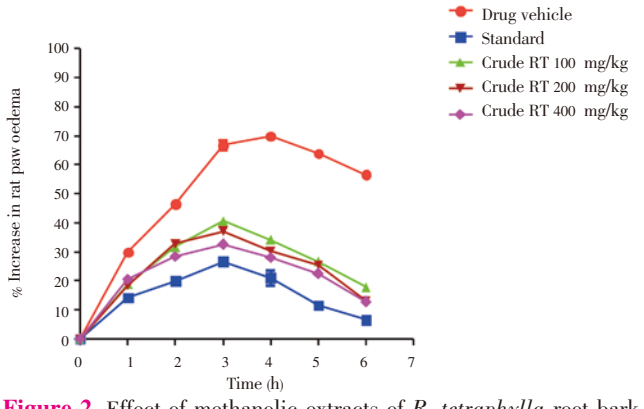

Figure 2. Effect of methanolic extracts of *R. tetraphylla* root bark, at 100, 200 and 400 mg/kg along with Indomethacin (2.5×10^{-5} moles/kg body weight).

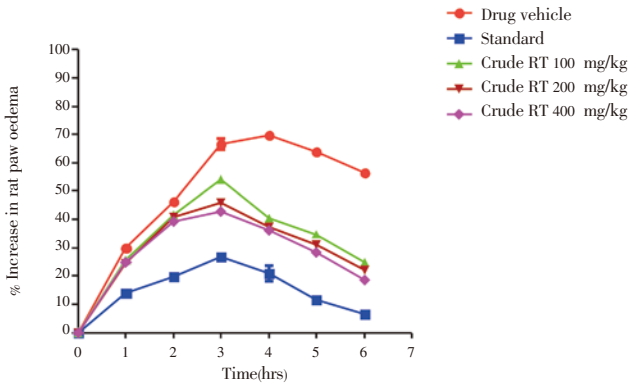

Figure 3. Effect of ethyl acetate extracts of *R. tetraphylla* root bark, at 100, 200 and 400 mg/kg along with Indomethacin (2.5×10^{-5} moles /kg body weight).

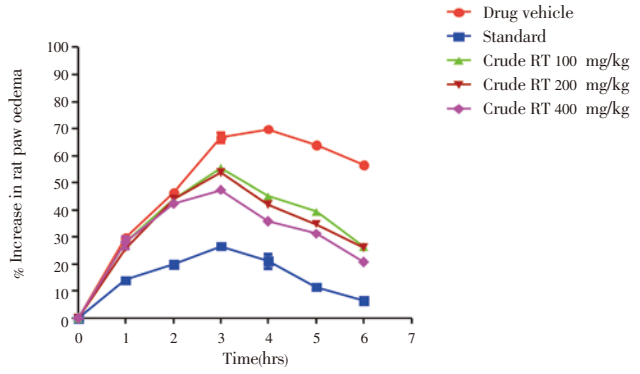

Figure 4. Effect of hexane extracts of *R. tetraphylla* root bark, at 100, 200 and 400 mg/kg along with Indomethacin (2.5×10^{-5} moles/kg body weight).

4. Discussion

All extracts at a concentration of 50 μg, 100 μg and 150 μg per cup exhibited antibacterial activity against tested bacterial strains in a dose dependent manner and showed significant activity when compared to that of the standard drug.

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury[9]. The development of oedema in the paw of the rat after the injection of carrageenan is due to the release of histamine, serotonin, prostaglandin and the like[10,11]. Acute hind paw oedema is induced in rats by injecting 0.1 mL of 1% v/v Carrageenan which reaches a peak edema levels at 3-5 hours after Carrageenan injection. Prostaglandin-E_2, a powerful vasodilator, synergizes with other infiammatory vasodilators such as histamine and bradykinin and contributes to the redness and increased blood fiow in areas of acute infiammation[12].

The above results clearly demonstrate that the extracts had significant and considerable anti-microbial activity against a variety of pathogens and produce significant anti-inflammatory activity. The results obtained in producing significant anti-inflammatory activity support the folkloric claims regarding the plants and their medicinal values. Perhaps people in the rural, tribal and forest areas have been constantly in touch with the value of plant products continuously throughout the centuries. Perhaps the modern world has lost touch with them due to various reasons.

Therefore, it appears to be an essential part of thinking to go back to plants for other medicinal, remedial and drug resistant extracts and application research. It may turn out to be highly beneficial to mankind in solving many problems associated with mans health.

Conflict of interest statement

We declare that we have no conflict of interest.

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