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Evaluation of Anti-Bacterial, Analgesic and Anti-Inflammatory activities of Oncocalyxone A isolated from *Prenanthes sarmentosus*

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ABSTRACT

The aim of the present study was to evaluate the anti-bacterial, analgesic and anti-inflammatory activities with oncocalyxone A isolated from the leaves of *Prenanthes sarmentosus*. The anti-bacterial activity of different concentrations of oncocalyxone A (100, 200 mg) was evaluated against four bacterial species, namely *Staphylococcus aurous*, *Escherichia coli*, *Aspergillus niger and Aspergillus flavus*. *Escherichia coli* showed the highest susceptibility to this compound. The oral dose of oncocalyxone A at a 200mg/kg exhibited analgesic and anti-inflammatory activities in comparison with standard drug Morphine sulphate at a dose of 5 mg/kg and Diclofenac sodium at a dose of 100 mg/kg respectively. The methanolic extract of *Prenanthes sarmentosus*, exhibited a weak anti-bacterial, analgesic and anti-inflammatory activities in comparison with isolated oncocalyxone A. The oncocalyxone A at dose of 200mg/kg showed highly significant activities as compared to standard drugs.

INTRODUCTION

This is an important medicinal plant commonly called as *Prenanthes sarmentosus* is a genus of plants in the family *asteraceae*, often referred to as Rattlesnake root (Ilayaraja *et al*., 2013). Natural products are often a source for bioactive compounds which have great potential for developing novel therapeutic agents (Hwang *et al*., 2000). A review of literature has revealed that plant metabolites such as alkaloids, flavonoids, glycosides, etc. play an important role in many of activities including wound healing, cardio-tonic, analgesic, anti-inflammatory, anti-oxidant, and antimicrobial activity (Prabakaran *et al*., 2013). These phytomedicine are not only cheap and affordable but are also safe. Infectious diseases account for high proportions of health problems in the developing countries. Micro organisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases (Rao *et al*., 2006). Nowadays, the use of antibiotics to control diseases is producing adverse toxicity to the host organs, tissues and cells. The toxicity produced by the antimicrobial agents can be cured or prevented or antagonized using herbs. Inflammation involves action of the complement system, blood coagulation, humeral and cellular immunity, cytokines, tissue hormones, angiogenesis, and repair processes. It is both a free radical generating and free-radical producing process (Miller, 1996).

A number of reports concerning the antibacterial, analgesics and anti-inflammatory activities of various plants have appeared in the literature, but the vast majority has yet to be explored. The aim of this study is to screen the anti-bacterial, analgesics and anti-inflammatory activities of oncocalyxone A isolated from the leaves of *Prenanthes sarmentosus*.

MATERIALS AND METHODS

Plant material

Prenanthes sarmantosus or Ehzutanippundu in Tamil was used as the test plant which was collected from rural area around Kumbakonam, Tamilnadu in the month of Feb - March and authenticated by Prof. N. Ramakrishnan, (Department of Botany) and voucher specimens (GACBOT-158) were deposited at the Herbarium of the Department of Botany, Government Arts College (Autonomous), Kumbakonam, Bharathidasan University, India.

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Extraction and Isolation

The important stage in the experimental work includes first the isolation of chemical substances from the chosen plant and secondly, the characterization of those isolated compounds. The fractions were collected such as $CHCl_3$ (66.0 g), EtOAc (22.5 g) and MeOH (27.4 g) and the solvent recovered by simple distillation. Structural elucidation of the compound isolated from $CHCl_3$ extract of *Prenanthes sarmentosus* leaves was accomplished by HPLC, UV, IR, and NMR spectroscopic methods. The hydro soluble components contained greater than 98 % of oncocalyxone A (Fig. 1) estimated from the ^1H NMR spectrum and HPLC analysis as reported the authors earlier (Ilayaraja *et al*., 2013).

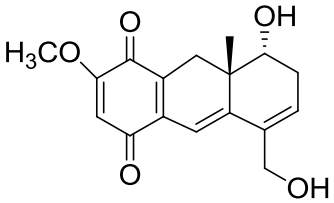

Fig. 1: structure of oncoxalyxone A.

Anti-bacterial activity by disc diffusion method

The 6 mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121 ℃. After the sterilization the moisture discs were dried on hot air oven at 50 ℃. Then various solvent extract discs and control discs were prepared. The bacterial strains of *escherichia coli* and *staphylococcus aureus and* fungal strains of *aspergillus flavus*, and *aspergillus niger* were obtained from Microbial Type culture Collection Centre (MTCC), Chandigarh. Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45 ºC.

The cooled media was added 10 ml/L tartaric acid (10%) act as antibacterial agents and poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various solvents extract prepared discs individually were placed on the each petriplates and also placed control and standard (Ciprofloxacin and Amphotericin) discs. The plates were incubated at 37 ºC for 24 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

Animals

Male albino mice (30-40g) and male albino rats (100-150 g) of Wistar strain were procured from the animal house, Department of Zoology, Government Arts College (Autonomous), Bharathidasan University, Kumbakonam, Tamilnadu, India. Animals were fasted overnight and were divided into control, standard and different test groups each consisting of six animals. They housed in cages and maintained under standard conditions at 26±2 ℃ and relative humidity 44-56% and 10 h light and 14 h dark cycles each day for one week before and during the experiments. All animals were fed with the standard rodent pellet diet, and water adlibitum. Before starting the experiment on animals, the experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee (IAEC), Bharathidasan University, Trichirappalli, Tamilnadu, India (Approval No. BDU/IAEC/2011/31/29.03.2011).

Analgesic activity

Hot-plate method

Hot-plate method was used in the current study (Abe *et al*., 1995; Al – Said *et al*., 1990). All groups were treated intraperitoneally, and each group received a particular treatment *i.e*. control (1% DMSO), positive control (Morphine sulphate 5mg/kg) and test samples groups received at a dose of 100, 200 mg of isolated oncocalyxone A and 300 mg/kg of the methanolic extract of *Prenanthes sarmentosus*.

All animals were lowered onto the surface of a hot plate (50 ±1.0 °C) enclosed with cylindrical glass and the time for the animal to jump or lick the fore limb was noted as the reaction time (RT). A cut off period of 30 seconds was observed to avoid damage to the paw. The observations were made before and after administration of respective drugs at 30 min, 60 min, and at the end of 120 min.

Anti-inflammatory activity

Carrageenan induced Rat paw edema

The anti-inflammatory activity of the test compounds were evaluated in Wistar rats employing the method (Diwan *et al*., 1989). The different test concentration of isolated oncocalyxone A and 300mg methanolic extracts of *Prenanthes sarmentosus* were administrated to the animals in the test groups at the dose of 100 and 200 mg/kg by oral route. Animals in the standard group received Diclofenac sodium at dose of 100 mg/kg, by oral route. Control group animals were received 1% DMSO at the dose of 10 ml/kg body weight. The acute inflammation was induced by the sub-plantar administration of 0.1 ml of 1% carrageenan in the right paw. Paw volume was measured by using digital plethysmometer (Ugo Basile-Italy) before administration of carrageenan and after 1, 2, and 3 hrs intervals (Kouadio *et al*., 2000). The efficacy of different drug was tested on its ability to inhibit paw edema as compared to control group.

Volume of edema = Final Paw Volume - Initial Paw Volume

The Percentage inhibition of paw edema was calculated by the formula as below.

% Inhibition of Paw edema = [(VC – VT) / VC] x 100

Where, VC = Paw edema of control group and VT = Paw edema of treated group

Statistical analysis

The experimental results were expressed as multiple comparisons of Mean ± SEM were carried out by one way analysis of variance (ANOVA) followed by Dunnet Multiple Comparisons Test and statistical significance was defined as $P< 0.05$.

RESULTS AND DISCUSSION

The isolation of oncocalyxone A (Fig. 1) from leaves of *Prenanthes sarmentosus* was subjected to column chromatographic separation analysis. Structure of the isolated compound was identified by HPLC and UV, IR, NMR spectroscopic methods were previously the authors reported (Ilayaraja *et al*., 2013).

Anti-bacterial activity

Anti-bacterial activity of plant origin is effective in the treatment of several infections. The action of compounds containing phenolic hydroxyl groups may be related to the inhibition of hydrolytic enzyme or other interactions to inactivate microbial adhesions (Vogel, 2008). In this study, the oncocalyxone A isolated from chloroform extract was studied for its anti-bacterial activity by using different clinically important strains at concentrations of 100 and 200 mg/disc by agar diffusion method. The microorganisms chosen to be studied were *Staphylococcus aureus, Escherichia coli*, *Aspergillus niger and Aspergillus flavus*. These bacteria were chosen to be studied as they are important pathogens and also due to rapidly developed antibiotic resistance as antibiotic use increases.

The activity of isolated oncocalyxone A was compared with the standard antibiotics, as mentioned in Table 1. In general, the mean zone of inhibition produced by the commercial antibiotic Ciprofloxacin and Amphotericin was between 11.0 and 24.0 mm and the inhibition produced by oncocalyxone A which was between 12.0 and 18.0 mm. The methanolic extract exhibit a inhibition zone between 8.0 and 11.0. Based on the results, the oncocalyxone A at a dose of 200 mg/ml showed the maximum zone of inhibition when compared with the commercial antibiotic against all the tested microorganisms. In a research conducted using the isolated compound from *Prenanthes sarmentosus* higher range of zone of inhibition against *Staphylococcus aureus* and *Escherichia coli* bacteria's at 200 mg/ml was found. However, the oncocalyxone A was also active against *Aspergillus niger* and *Aspergillus flavus.*

Table 1: Anti-bacterial activity of oncocalyxone A from *Prenanthes sarmentosus*

S. N.	Micro organisms	Zone of inhibition mm in diameter (M±SD)			
		Oncocalyxone A		Methanolic Extract (300 mg)	Standard
		100 mg	200 mg		
1	*Escherichia coli*	14 ± 1.20	18 ± 1.45	11 ± 1.16	20 ± 1.22*
2	*Staphylococcus aureus*	12 ± 1.14	16 ± 1.16	9 ± 1.19	24 ± 1.45*
3	*Aspergillus niger*	9 ± 1.21	12 ± 0.82	7 ± 0.86	14 ± 0.98**
4	*Aspergillus flavus*	11 ± 1.14	14 ± 1.02	8 ± 1.08	11 ± 0.67**

Bacteria Standard* - Ciprofloxacin (5 mg);
Fungal Standard****** - Amphotericin – B (20 mg)
Values are expressed in Mean ± Standard Deviation (M±SD) (n=3)

Analgesic activity

The analgesic activity of different dose of 100, 200 mg of isolated oncocalyxone A and 300 mg/kg of the methanolic extract of *Prenanthes sarmentosus* by hot plate method, it was observed that analgesic effect at 30, 60 and 120 minutes. The analgesic activities are comparable with the reference analgesic agent (Morphine sulphate) used in the present study with significant increase in the reaction time in comparison with the control group. The isolated oncocalyxone A from *Prenanthes sarmentosus* showed significant increase in time latency to heat stimulus as compared with control group, also Morphine sulphate induced an increase in time latency of pain (Table 2).

The hot plate induced pain test was performed in order to determine whether the analgesic activity of the extracts was caused by central or peripheral mechanisms, where the hot plate test is believed to show the involvement of central mechanisms (Collier *et al*., 1968). Normal 1% DMSO solution (control group) did not have any significant change in reaction time periods. The oncocalyxone A at dose 200 mg/kg showed a significant activity at 30 minute and highly significant activity at 60 and 120 minutes. As compared to standard drug, the methanolic extract at a dose of 300 mg/kg was found to have no significant differences at different time periods. The methanolic extract at a dose of 300 mg/kg showed peak effect 11.3 ± 0.19 at 120 minutes.

Table 2: Analgesic activity of oncocalyxone A from *Prenanthes sarmentosus*

Groups	Treatment	Dose (mg / kg)	Reaction Time (in minutes)		
			30	60	120
I	1% DMSO	10	5.2 ± 0.20	5.24 ± 0.202	5.34 ± 0.204
II	Morphine sulphate	5	9.6 ± 0.11	12.5 ± 0.12	13.7 ± 0.09
III	Methanolic extract	300	6.8 ± 0.21	10.2 ± 0.197	11.3 ± 0.19
IV	Oncocalyxone A	100	8.2 ± 0.15	11.4 ± 0.146	12.0 ± 0.145
V	Oncocalyxone A	200	9.3 ± 0.1	12.1 ± 0.08	13.4 ± 0.11

Values are expressed in Mean ± Standard Deviation (n=6)
One-way ANOVA (Dunnetts method) Means for groups in homogeneous subsets are displayed.
Subset for alpha = 0.05 level.

Anti-inflammatory activity

The anti-inflammatory activities of two different concentrations (100 and 200 mg) of oncocalyxone A and 300 mg methanolic extracts of *Prenanthes sarmentosus* were assessed by carrageenan induced paw edema method. Carrageenan induced paw edema is suitable experimental animal model for evaluation anti- edematous effect of natural products (Winter et *al*., 1962). The previous reports that the carrageenan induced paw edema takes place in three phases, in the first phase (1 hr after carrageenan induce) involves the release of serotonin and histamine from mast cells, in second phase (2 hrs) is provided by kinins and the third phase (3 hrs) is mediated by prostaglandins, the cycloxygenase and lipoxygenase products (Vinegar *et al*., 1969). The methanolic extract of *Prenanthes sarmentosus*, exhibited a weak inhibitory effect on paw edema volume with percentage inhibition of (33.61%) compared to control (Table 3). It may be attributed to the fact that the plant extract being in crude form contains a smaller concentration of bioactive compounds. Oncocalyxone A at a dose of 200 mg/kg showed highly significant anti-inflammatory activity as compared to control group at 1, 2 and 3 hours respectively. The Oncocalyxone A at 100 mg/kg was found to have significant activity at 3 hour. The standard drug

Diclofenac sodium at a dose of 100 mg/kg body weight inhibited the development of edema significantly from 1 hour onwards. It showed maximum percentage reduction (66.60%) in paw edema at 3 hour. Oncocalyxone A at the dose of 100 and 200 mg/kg body weight showed percentage of inhibition of paw edema at 3 hour 40.33% and 53.78% respectively.

Table 3: Anti - inflammatory activity of oncocalyxone A from *Prenanthes sarmentosus*

Groups	Treatment	Dose (mg / kg)	Inflammation in cm (M±SD)		
			1 h	2 h	3 h
I	1% carrageenan	10	3.60 ± 0.04	3.58 ± 0.15	3.57 ± 0.15
II	Diclofenac sodium	100	2.12 ± 0.08	1.72 ± 0.10	1.19 ± 0.15
III	Methanolic extract	300	2.89 ± 0.03	2.60 ± 0.12	2.37 ± 0.13
IV	Oncocalyxone A	100	2.64 ± 0.03	2.32 ± 0.08	2.13 ± 0.07
V	Oncocalyxone A	200	2.38 ± 0.10	2.09 ± 0.06	1.65 ± 0.15

Values are expressed in Mean ± Standard Deviation (n=6)
One-way ANOVA (Dunnetts method) Means for groups in homogeneous subsets are displayed.
Subset for alpha = 0.05 level.

CONCLUSION

On the basis of the present study, it is concluded that the oncocalyxone A isolated from *Prenanthes sarmentosus* scientifically justifies the use in the folklore remedies for anti-bacterial, analgesics and anti-inflammatory activities. The methanolic extract of *Prenanthes sarmentosus*, exhibited a weak anti-bacterial, analgesic and anti-inflammatory activities in comparison with isolated oncocalyxone A. The oncocalyxone A at dose of 200 mg/kg showed highly significant activities as compared to standard drugs.

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