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RESEARCH ARTICLE

In Vitro anti-inflammatory activity of Various Extracts from *Trianthema decandra* Linn.,

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Abstract:
Trianthema decandra Linn. belongs to family Aizoaceae, commonly known as "Gadabandi" in Hindi & "Punarnani" in Sanskrit. This plant is globally distributed in Indo-Malaysia, Burma and Australia. Within India, it is found in the Rajasthan, Uttar Pradesh up to Haryana. By looking the high traditional use of the plant *Trianthema decandra* Linn, The crude extract of the whole plant has been reported to be superior as a wound dressing material. The leaf extract has been used in the treatment of chronic pain of osteoarthritic patients. This plant is mainly used in the as analgesic, antimicrobial and inflammation. Preliminary Phytochemical screening reported the presence of alkaloids, phytosterols, flavonoids, saponins, tannins and phenolic compounds in the chloroform and methanol extracts of the plant. For screening the anti-inflammatory activity we used In-vitro method i.e. HRBC membrane stabilizing activity. In this method we compare the HRBC membrane stabilizing activity for both the chloroform and methanol extract, the Methanolic extract showed the better activity than the Chloroform extract. Both the extracts showed statistically significant ($P<0.01$) values in the dose dependent manner when compared with standard Diclofenac sodium. At 500µg and 400µg the percentage protection of methanolic extract and standard Diclofenac sodium was found to be 72.15 ± 1.58, 71.20±0.89, 75.63±0.81 & 71.51 ± 0.82 respectively.

Keywords: *Trianthema decandra*, Chloroform and methanol extracts, HRBC membrane stabilizing activity, Diclofenac sodium.

Introduction

Trianthema decandra Linn., belongs to family Aizoaceae. Commonly known as gadabani (Hindi) and vellai sharuni (Tamil) is a prostrate herb distributed in the tropical and sub-tropical regions of the world and also found abundantly in India[1][2]**.** The inflammatory response is the body's natural response that occur immediately following tissue damage. Its main functions are to defend the body against harmful substances, dispose of dead or dying tissue and to promote the renewal of normal tissue. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury and pain. Even though most of the synthetic anti-inflammatory drugs are available in the market, due to their well-known side effects, toxic effects and production cost, presently people are search for natural anti-inflammatory drugs without any adverse effects[3][4].

HRB or erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of human red blood cell membrane lyses can be taken as an in vitro measure of anti-inflammatory activity of the plant extracts[5][6][7],.

OBJECTIVE:
The objective of present study is to evaluate anti-inflammatory activity using HRBC membrane stabilizing method.

EXPERIMENTAL METHODS:
Extraction:
The fresh whole plant parts of *Trianthema decandra* was collected and authenticated by Dr. K.Madhava Shatty, Assitant Professor, Dept. of Botany, S.V. University, Tirupathi, A.P.[8] whereas the dried plant parts powder was subjected to extraction by sotxhlet apparatus using various solvents ranging from non polar to polar and obtained extracts used for determination of phytochemical analysis[9].

Preliminary Phytochemical screening :
The chloroform, acetone, methanol and water extracts were subjected for chemical tests for the identification of active constituent by using methods of Kokate (1996) and khandelwal (2005) (Table No. 1).

Procedure:
The HRBC membrane stabilization has been used as a method to study the anti-inflammatory activity. Human blood was mixed with equal volume of sterilized Alsever solution. Alsever solution contains dextrose, sodium citrate, sodium chloride in water. The blood was centrifuged and 10% v/v suspension was made with Isosaline. The drug samples were prepared by suspending the residues in hot water. The assay mixture contained the drug, 1 ml phosphate buffer, 2 ml hypo saline, 0.5 ml HRBC suspension and Diclofenac sodium was used as the reference drug. Instead of hypo saline 2 ml of distilled water was used in the control. All the assay mixture were incubated at 37^0 C for 30 minutes and centrifuged. The hemoglobin content in the superannuated solution was estimated using spectrophotometer at 560 nm. The percentage of hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100 %[10].

The percentage of HRBC membrane stabilization was calculated using the formula.

Percentageprotection=

$$100 - \frac{\text{Optical density of drug treated sample}}{\text{Optical density of control}} \times 100$$

Statistical analysis:
Experimental data were expressed as mean ±SD. The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA) followed by Dunnet Multiple comparison test (standard vs. test) using the software Graph Pad In stat. The differences were considered to be statistically significant when $P<0.05$.

RESULTS AND DISCUSSION:

Preliminary Phytochemical screening:

In preliminary Phytochemical screening carbohydrates, alkaloids, phytosterols, saponins flavonoids, tannins & phenolic compounds were reported in the chloroform and methanol extract and in the aqueous extract glycosides were reported.

HRBC MEMBRANE STABILISING ACTIVITY:

Comparative anti inflammatory activity for chloroform and methanol extract of *Trianthema decandra* Linn by HRBC membrane stabilization method.

In the HRBC membrane stabilizing property, extracts showed significant anti-inflammatory activity in a concentration depended manner. Methanol extract at a concentration of 500 µg/ml showed 72% protection of HRBC in hypotonic solution compared with standard Diclofenac sodium which showed 75% protection and methanol extract at 400 µg/ml concentration showed same percentage of protection of HRBC in hypotonic solution as compared with standard i.e 71%.

Conclusion:

The In-vitro anti-inflammatory activity is moderate for both the extracts. The anti-inflammatory activity which is reported is mainly because of the secondary metabolites like the Alkaloids, Steroids, Saponins, Flavonoids, Tannins and Phenolic compounds which has well established claim for the anti-inflammatory activity.

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Table No. 1: Preliminary Phytochemical screening:

Compounds	Chloroform	Acetone	Methanol	Water
Carbohydrates	_	_	+++	+
Glycosides	_	_	+	+
Steroids	+++	++	+	_
Proteins	_	_	_	+
Phenolic compounds	_	_	++	+
Flavonoids	++	+	_	_
Alkaloids	++	+	++	+
Fats and oils	_	_	-	-
Saponins	_	+	+	+

+++ Indicates High conc., ++ Indicates Moderate conc., + Indicates Low conc., - Indicates Absent.

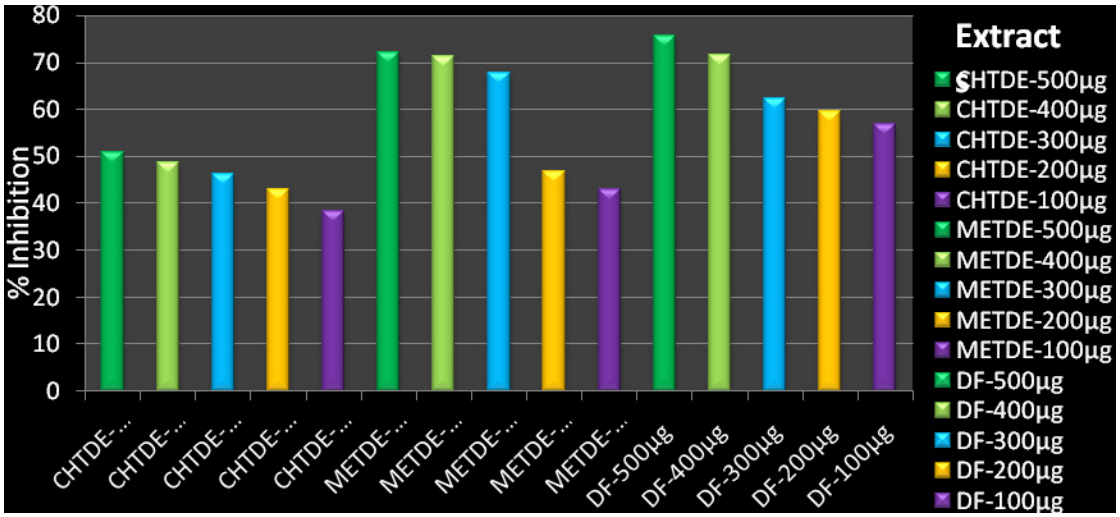
Table 2:

Treatment	Concentration (µg/ml)	Absorbance	% Inhibition
Control	------	0316 ± 0.011	-------
CETD	500µg	0.155±0.029	50.94 ± 1.12
	400µg	0.162 ± 0.012	48.73 ± 1.89
	300µg	0.170 ± 0.031	46.20 ± 1.24
	200µg	0.180 ± 0.024	43.03 ± 2.86
	100µg	0.195 ± 0.021	38.29 ± 0.92
METD	500µg	0.088 ± 0.021	72.15 ± 1.58
	400µg	0.091 ± 0.031	71.20 ± 0.89
	300µg	0.102 ± 0.112	67.72 ± 0.56
	200µg	0.168 ± 0.014	46.83 ± 1.21
	100µg	0.180 ± 0.018	43.03 ± 0.98
DF	500µg	0.077 ± 0.022	75.63 ± 0.81
	400µg	0.090 ±0.011	71.51 ± 0.82
	300µg	0.119 ± 0.012	62.34 ± 1.22
	200µg	0.128 ± 0.011	59.49 ± 1.14
	100µg	0.136 ± 0.013	56.96 ± 0.19

Each value expresses mean ± SD, n=3, ** P<0.01, * P<0.05

Figure 1

Fig.15. Graph representing the effect of *Trianthema decandra Linn.* on In-vitro anti-inflammatory activity by HRBC membrane stabilization.



n=3** represents P<0.01, * represents P<0.05

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