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INVITRO ANTI-ARTHRITIC ACTIVITY OF CROSSANDRA INFUNDIBULIFOMIS LEAF EXTRACT

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ABSTRACT

The present study have been designed to evaluate the *In-vitro* Anti-Arthritic activity of herbal plant *Crossandra infundibuliformis* belonging to the family Acantheacea. The leaves were collected, dried and extracted by soxhlet with solvents like Methanol, Petroleum Ether. The inhibition of protein denaturation by Egg-Albumin method was taken as a measure of the *in-vitro* anti-arthritic activity. The percentage inhibition of protein denaturation is obtained as 89.4%, 91.2% and 94.3% for petroleum ether extract and 81.8%, 84.3%, 88.5% for methanol extract respectively at a dose of 100, 250, 500 µg/ml. The percentage inhibition of standard diclofenac sodium was found out to be 91.2%, 94.5% and 96.4% respectively at a dose of 100, 250, 500 µg/ml. Pet.ether extract was found to be more effective than Methanolic extract.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease predominantly affect the joints and particular tissue. Rheumatoid arthritis still remains a formidable disease, being capable of producing severe crippling deformities and functional disabilities and cartilage destruction and commonly leads to significant disability, caused by number of pro inflammatory molecules released by microphages including reactive oxygen species and eicosanoids such as prostaglandins, leukotrienes and cytokines.[1] The regulation of these mediators secreted by microphages and other immune cells and modulation of arachidonic acid metabolism by inhibiting enzymes like COX and LOX are the potential target for

chronic inflammatory conditions. Rheumatoid arthritis is a complex process, involving synovial cells proliferation and fibrosis, pannus formation and cartilage and bone erosion.[2] This process is mediated by an inter-independent network of cytokines, prostanoids and proteolytic enzymes.

Majority of human population worldwide is getting affected by the inflammation related disorders. There are four main groups of drugs used to treat arthritis: pain killers (analgesics), Non-Steroidal Anti-Inflammatory Drugs (NSAIDS), Disease Modifying Anti Rheumatic drugs (DMARDS) and Corticosteroids (steroids). There are many synthetic drugs that are being used as standard treatment for rheumatoid arthritis but they have adverse effects that can compromise the therapeutic treatment so this adverse effects increase the chances for the uses of herbal plants for the rheumatoid arthritis treatment.[3]

Herbal drugs constitute a major part in all the traditional system of medicines herbal medicine is a triumph of popular therapeutic diversity. The factors responsible for the continued and extensive use of herbal remedies in India are their effectiveness, easy availability, low cost, comparatively less toxic effects and shortage of practitioners of modern medicine in rural areas.[4]

MATERIALS AND METHODS

Collection of leaves and authentification

The fresh leaves of *crossandrainfundibuliformis* (*Acantheceae*) were collected from village peddasettypalli, proddutur, Kadapa district, Andhra Pradesh, India in the plant was authenticated by D. VASU BABU. The leaves were and the shade dried at room temperature and the shade dried leaves of crossandrainfundibuliformis were powdered 40 mesh size.

Extraction of leaves of *Crossandra infundibuliformis*

The powdered material of plant was passed through 40 mesh size. The dried powdered(50g) was extracted with methanol and petroleum ether using soxhalet apparatus for about 72 hours. After extraction with solvent, the marc was dried in hot air oven below 40^0 c and was concentrated by distilling off the solvent to evaporate to dryness,[5] The dried extract was subjected to preliminary, phytochemical screening for detection of various phytoconstituents.

Phytochemical studies

Name of the test	Indication
Flavonoids	+ve
Terpenoids	+ ve
Tannins & phenolic compounds	+ ve
Steroids	+ ve
Alkaloids	+ ve
Cardiac glycosides	+ ve
Reducing sugars	+ ve
Saponins	+ ve

Preparation of Egg Albumin

Procedure

Collect egg white from eggs carefully, avoiding the egg yolk, into a 500 ml beaker. Dilute the egg white to 100 ml by adding distilled water with vigorously beating and stirring.[6]

Precipitate of ovomucoid is removed by centrifugation.

⇩

Solid ammonium sulphate is added (32.5 g)

⇩

Stirr gently at room temperature with (avoid frothing).

⇩

Keep aside the contents for 15 minutes at room temperature.

⇩

Precipitate is formed which is of globulin

⇩

It is removed by filtration or centrifugation

⇩

Saturate the supernatant by adding 35.5gm solid ammonium sulphate

⇩

Stirr the contents at the room temperature for 30 minutes

⇩

Recovery of Albumin precipitate by centrifugation

Dissolve the precipitate in minimum volume of distilled water

⇩

Dialysis the protein solution extensively cold water to remove the salt

⇩

A white precipitate of barium sulphate is observed.

⇩

Measure the volume of protein solution after dialysis

⇩

Add 0.05%w/v sodium oxide as preservative

Preparation of test sample

Samples for experiments were prepared by dissolving extract to obtain a stock solution of 100mg/ml, from stock solution, different working dilutions were prepared to get concentration range of 100, 200, 300 mg/ml of petroleum ether extracts. For present study diclofenac sodium taken as standard drug.[7] The concentration of standard drug was prepared in 100, 250 ,500 mg/ml of concentration.

Phosphate buffer saline p^H 6.3

Dissolve the 8 gms of sodium chloride(Nacl), 2.2 gms of potassium chloride(KCL), 1.44 gms of dihydrogen phosphate(Na_2PHO_4), 0.4 gms of potassium dihydrogen phosphate (KH_2PO_4) in 800 ml of distilled water. The p^H was adjusted to 6.3 using 1N HCL and make up the volume 1000 ml with distilled water.[8]

Percentage inhibition = (Abs sample - Abs control) x Abs sample

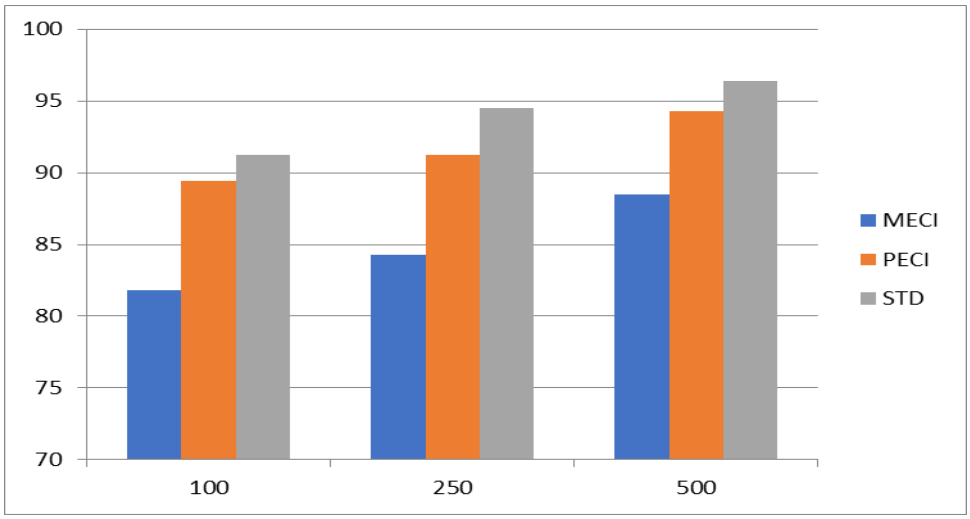
Abs = Absorbance

RESULTS

S. no.	Plant constituent	Test	*Cro1ssandra infundibuliformis*
1.	Carbohydrates	Molish's reagent	-ve
2.	Amino acids	Ninhydrin test	-ve
3.	Sterols	Salkowski reaction Libermann- Burchard reaction[9]	+ve +ve
4.	Tannins	Ferric chloride soln test	+ve
5.	Phenolic compounds	Lead acetate test Dilute iodine test	+ve +ve
6.	Saponin glycosides	Forth formation test	+ve
7.	Flavanoids	Shinoda test Alkaline reagent test Zinc hydrochloride test.[10]	+ve +ve +ve
8.	Alkaloids	Dragendroff's reagent Mayer's reagent Wagner's reagent	+ve +ve +ve

Where +ve = Positive, -ve = Negative

S. No.	Groups	Concentration (mg/ml)	Absorbance (nm)	% inhibition
1.	Control	_	0.040	_
2.	Diclofenac	100	0.455	91.2%
		250	0.735	94.5%
		500	1.130	96.4%
3.	MECI	100	0.220	81.8%
		250	0.255	84.3%
		500	0.350	88.5%
4.	PECI	100	0.380	89.4%
		250	0.455	91.2%
		500	0.711	94.3%



X-Axis: Concentration of Standard and sample extracts

Y-Axis: Percentage inhibition of protein denaturation

MECI: Methanolic extract of *Crossandra infundibuliformis* leaf extract

PECI: Petroleum ether extract of *Crossandra infundibuliformis* leaf extract

CONCLUSION

Inhibition of protein denaturation was studied to establish the mechanism of anti arthritic effect of petroleum ether extract of C*rossandra infundibuliformis* leaves.[11] Therefore, our present invitro studies on petroleum ether extract of *Crossandra infundibuliformis* leaves demonstrated the significant anti arthritic activity. Due to the presence of active principles such as terpenoids, cardiac glycosides, flavanoids may responsible for this activity.[12]

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