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INVITRO EVALUATION OF ANTI-ARTHRITIC ACTIVITY IN METHANOLIC EXTRACT OF *CONOCARPUS ERECTUS*

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ABSTRACT

Objective: *Conocarpus erectus* belongs to the family Combretaceae and traditionally it is claimed to be used in the treatment of anemia, conjunctivitis, gonorrhoea, diabetes, diarrhea, fever, headache, bleeding, tumors, orchitis, prickly heat, swellings, and syphilis.

Materials and Methods: In the present study, methanolic extract of leaves of *Conocarpus erectus* was subjected to phytochemical screening for the presence of phytoconstituents. Methanolic extract of leaves of *Conocarpus erectus* showed the presence of phenols, tannins and flavonoids. These extract was then taken up for the study of in-vitro anti arthritic activity by inhibition of protein denaturation was taken as a measure of the activity.

Results & Conclusion: The maximum percentage inhibition of protein denaturation for *Conocarpus erectus* leaves extracts were found to be 75.47%, 84.22% and 86.36% respectively at a dose of 600, 800, 1000 µg/ml. When compared to standard diclofenac sodium was found out to be 84.21%, 87.52% and 90.81% respectively at a dose of 600, 800, 1000 µg/ml. Therefore, our studies support the isolation and the use of active constituents from *Conocarpus erectus* leaves in treating arthritis.

Key Words: *Conocarpus erectus* leaves, invitro anti-arthritic activity, diclofenac sodium.

INTRODUCTION

Arthritis is an autoimmune disorder characterized by pain, swelling and inflexibility [1]. Rheumatoid arthritis may rapidly progress into a multisystem inflammation with irreversible joint destruction and increase the risk of mortality [2]. It is an inflammation of synovial joint due to immune mediated response. Recently, it has been reported that microorganism including bacteria, viruses, fungi, parasites, bacterial DNA, and bacterial toxin may exacerbate the inflammatory response in the joint and bone [3]. Pathophysiology of exaggerated synovial tissue, involves hyperplasia and subintimal infiltration of T and B. lymphocytes, this result in pannus tissue that irreversibly destroys the cartilage and bone in the affected joint. Polymorphonuclear leukocytes and macrophages are also stimulated which result in the production of inflammatory mediators including large

amount of superoxide and hydrogen peroxide that can cause significant impairment and destruction of synovial fluid, cartilage and other articular constituents [4, 5]. The production of auto antigens in certain arthritic diseases may be due to *in vivo* denaturation of proteins [6]. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding [7]. So, by controlling the production of auto antigen and inhibiting denaturation of protein in rheumatic disease leads to anti-arthritic activity. Hence, inhibition of protein denaturation was taken as a measure of the *invitro* anti-arthritic activity [8].

Conocarpus erectus belongs to the family Combretaceae and traditionally it is claimed to be used in the treatment of anemia, catarrh, conjunctivitis, gonorrhoea, diabetes, diarrhea, fever, headache, bleeding, tumors, prickly heat, swellings, and syphilis.

Conocarpus erectus L. is commonly known as Button mangrove, Buttonwood. *C. erectus* L. a plant used for road-side medians, parking lots, screening hedges, landscaping purposes as well as folklore medicine [9].

MATERIALS AND METHODS

Collection: *Conocarpus erectus* leaves collected from Proddatur, Andhra Pradesh, India. The leaves were separated and washed thoroughly in running tap water to remove soil particles and other adhered debris and then finally washed with sterile distilled water. The leaves were air dried.

Preparation of Defatted Methanol Extracts:

Two hundred grams of finely *Conocarpus erectus* leaves plant powder soaked in 1000 ml methanol for one week at room temperature with shaking day by day followed by filtration and again extraction for four times. The organic solvent was removed using rotator evaporator under vacuum affording known weight of each crude methanol extract. The methanolic crude extract was defatted by washing several times with petroleum ether (60-80°C). The defatted extract was ready for activity.

Phytochemical screening:

Phytochemical screening the methanol extract of this plant were screened for the presence of various phytochemical constituents such as alkaloids, flavonoids, phlobatannins, anthroquinones, steroids, tannins, phenols, terpenoids, saponins, resins, carbohydrate and protein and amino acids compounds [10].

Evaluation of *invitro* anti-arthritic activity

Protein denaturation by using egg albumin [11]

Each reaction mixture consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of various solvent extracts of *Conocarpus erectus* so that final concentrations become 100, 200, 400, 600, 800 and 1000 µg/ml. Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37±2) °C in a Biochemical oxygen demand (BOD) incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm.

Diclofenac sodium (100, 200, 400, 600, 800 and 1000 µg/ml) was used as reference drug. The percentage inhibition of protein denaturation was calculated by using the following formula.

Percentage inhibition =

(Abs of sample – Abs of control) X 100/ Abs sample

Whereas Abs- Absorbance.

Statistical analysis

The data was analyzed statistically using ANOVA followed by student't' test with GraphPad Prism Data Editor for Windows, Version 6.0 (GraphPad software Inc., San Diego, CA). Values were expressed as mean ± Standard error for mean (± SEM). P < 0.05 - 0.01 were considered as statistically significant.

RESULTS

Table 1: The Phytochemical constituents of methanolic extract of the *C. erectus*.

S.No.	Plant constituent	Test	Methanolic extract of <i>CONOCARPUS ERECTUS</i> leaves
1	Carbohydrates	Molish's reagent	+ve
2	Amino acids	Ninhydrin test	+ve
3	Sterols	Salkowski reaction Liebermann – Burchard reaction	+ve
6	Tannins & Phenolic compounds	Ferric chloride solution test Lead acetate test Dilute Iodine test Dilute HNO ₃ test Acetic acid test Dilute KMNO ₄ test	+ve +ve +ve +ve +ve +ve
7	Saponin glycosides	Froth formation test	+ve
8	Flavonoids	Shinoda test Alkaline reagent test Zinc hydrochloride test	+ve +ve +ve
9	Alkaloids	Dragendroff's reagent Mayer's reagent Wagner's reagent Saturated picric acid test	+ve +ve +ve +ve

Table 2: Invitro antiarthritic effect of methanolic extract of *Conocarpus erectus leaves*

S.NO.	Groups	Concentration (mg/ml)	Absorbance (nm)	% inhibition
1	Control	-----	0.003±0.002	-----
		100	0.007±0.003	57.14±3.70
		200	0.011±0.004	72.72±3.52
2	Diclofenac	400	0.015±0.003	80.00±2.63
		600	0.019±0.004	84.21±1.63
		800	0.024±0.003	87.52±1.08
		1000	0.0321±0.003	90.81±0.80
		100	0.006±0.003	50±3.39
3	MECE	200	0.008±0.003	62.51±2.73
		400	0.011±0.001	72.71±2.16
		600	0.016±0.001	75.47±2.92
		800	0.019±0.002	84.22±1.33
		1000	0.022±0.002	86.36±1.21

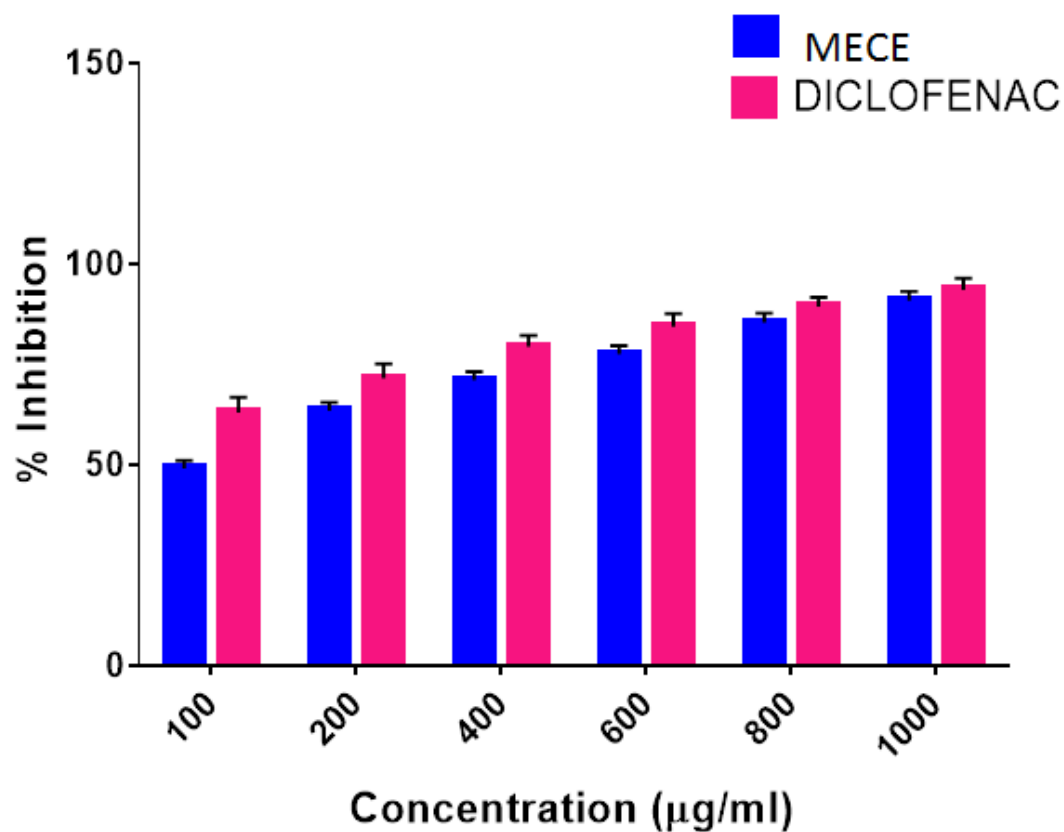


Figure No. 1: Invitro ant arthritic activity of diclofenac and methanolic extract of *Conocarpus erectus leaves* by protein denaturation method comparing Concentration Vs % Inhibition

DISCUSSION

Phytochemical screening of methanolic extract of *C. erectus* was used to study the presence of phytoconstituents: alkaloids, flavonoids, steroids, phenolic compounds, saponins, & tannins and also have various medicinal values such as anti-inflammatory, anti-oxidant, anti-diabetic, anti-microbial and analgesic activities. The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and antibiotic properties. Steroids and triterpenoids show the analgesic properties. The steroids and saponins were responsible for central nervous system activities [12].

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. For example, enzymes lose their activity, because the substrates can no longer bind to the active site [13]. Denaturation of protein is one of the cause of rheumatoid arthritis was documented. Production of auto antigen leads to denaturation of protein in certain arthritic disease. Modulation of electrostatic, hydrogen, hydrophobic and disulphide bonding in denaturation of protein, which is the mechanism of protein denaturation. This anti-denaturation effect was further supported by the change in viscosities. It has been reported that the viscosities of protein solutions increase on denaturation [14,15].

From the result of the present study, it can be stated that all the extracts of *C. erectus* are capable of controlling the production of auto – antigen and thereby it inhibit the denaturation of proteins of both fresh egg albumin in dose dependent manner and its effect was compared with the standard drug diclofenac sodium. Methanol extract of *C. erectus* showed maximum inhibition of protein denaturation. The activity may be due to the presence of phytocompounds with anti-arthritic activity.

CONCLUSION

Inhibition of protein denaturation was studied to establish the mechanism of anti-arthritic effect of Methanolic extract of *Conocarpus erectus* leaves. Therefore, our present in-vitro studies on Methanolic extract of *Conocarpus erectus* leaves demonstrated the significant anti-arthritic activity.

Due to the presence of active principles such as flavonoids, tannins and phenolic compounds may responsible for this activity.

Further studies are necessary to isolate and reveal the active compound contained in the crude extracts of *Conocarpus erectus* leaves responsible for activity

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