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In vitro Anti-arthritic and Hemolytic Activity of Leaf Extracts of *Tragia involucrate*

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Abstract: *Tragia involucrata* commonly known as Indian stinging nettle is a shrub widely used in traditional ayurvedic system for treatment of cephalagia, skin infections, eczema, and bronchitis. However, in use as a traditional medicine since ancient time, its anti-arthritic and hemolytic properties are not yet studied. The present study aimed to assess the phytochemical analysis, *in vitro* anti-arthritic and hemolytic activity of the petroleum ether, chloroform, ethyl acetate and aqueous extracts of *Tragia involucrate.* The phytochemical analysis reveals the presence of phytoconstituents such as phenols, flavanoids, sterols and terpenoids etc. The maximum inhibition of anti-arthritic activity of 93% at the concentration of 200µg/ml was observed in chloroform extract. The hemolytic activity of the extracts on human erythrocytes was performed by standard method and the extracts were found to be non-hemolytic against the red blood cell membranes.
Keywords: *Tragia involucrata*, Phytochemical analysis, Anti-arthritic, Hemolytic.

Introduction and Experimental

Rheumatoid arthritis is a progressive autoimmune disorder characterized by chronic inflammation and hypertrophy of synovial membranes, [1] that ultimately leads to destruction of the bones, joints and articular cartilages. [2, 3] The pathology of rheumatoid arthritis is very complex and the reason underlying the mechanism also remains unknown. Arthritis can affect everyday task of a person, leading to severe disability and may cause premature deaths.[4] The conventional drugs used for the treatment of this disease such as non-steroidal anti-inflammatory agents, immunosuppressants and corticosteroids to newer biological molecules such as monoclonal antibodies and TNF-α, the treatment failed to give long term effects with adverse side effects like ulcer, cardiovascular complications and nephro and hemato toxicity.[5,6] Thus, there is a need to find alternative method for treatment of this complex disorder hence, the complementary and alternative medicines traditionally used can meet the requirements of patients suffering from arthritis.[7, 8]

Tragia involucrata is a perennial herb belonging to the member of Euphorbiaceae family found in India and Asian pacific regions. It is commonly known as kanchori.[9] Traditionally, the leaves are used by rural community for treatment of skin infections, pain, swelling, eczema, [10] jaundice treatment by the chakma ethnic group in Bangladesh[11] and the root extracts of the plantis used in relieving bronchitis, attendant fever[12] and also as an external applicant in case of leprosy[13]. Phytochemical analysis reports revealed the presence of terpenoids, sterols, phenol and flavanoid.[14] Several reports state that plant is having potent antimicrobial,[15] anti-fertility, [16] anti-inflammatory,[17] analgesic,[18] nephroprotective [19] antipyretic and wound healing properties.[20]

Hence, in the present study, we attempted to evaluate the *in vitro* anti-arthritic and hemolytic effect of aqueous, ethyl acetate, chloroform, and petroleum ether extracts of *T. involucrata* leaves.

Chemicals

The chemicals and solvents used for extraction, phytochemical analysis were purchased from SD Fine chemicals Ltd., Mumbai, India.

Plant collection

The leaves of *T. involucrata* were harvested from Vellore district, Tamil Nadu, India in September 2013. Dr. P.K. Jayaraman, Plant Anatomy Research Centre, Chennai carried out authentication of the leaves and a voucher specimen has been preserved for further references.

Preparation of extracts

The fresh leaves of *T. involucrata* were washed with distilled water, shade dried, and pulverized. 50g of powdered sample was successively extracted with different solvents in order of their polarity like petroleum ether (TI-P), chloroform (TI-C), ethyl acetate (TI-E) and aqueous (TI-A) by soxhlet method. The extracts was concentrated under reduced pressure using a rotary evaporator and stored at 4^0C for further studies.

Phytochemical Screening

The preliminary phytochemical analysisof TI-P, TI-C, TI-E, and TI-A extracts of *T. involucrata* for the presence of constituents like alkaloids, carbohydrates, phenols, terpenoids, phytosterols, flavanoids and proteins was performed using standard procedures as described in J.B. Harbon. [21]

***In vitro* Anti-arthritic activity**

The anti-arthritic property of the leaf extracts of *T. involucrata* was studied by protein denaturation inhibition assay[22] . Diclofenac sodium was used as a standard.The test solution consists of 2 ml of various concentrations (50-200 µg/ml) of extracts, 0.2 ml of fresh egg albumin and 2.8 ml of phosphate buffer saline solution of pH 6.4. The control solution consists of 0.2 ml of egg albumin and 2.8 ml of phosphate buffer saline solution. The mixtures were incubated at 37°C for 30 min and furtherat an increased temperature of 57°C for 10 min. The absorbance of the sample was measured using UV-Visible spectrophotometer at 600 nm.

% Inhibition = Ac – At /Ac x 100

Where,"Ac" is the absorbance of control and "At" is the absorbance of the test sample. The experiment was performed in triplicates.

Hemolytic activity

The TI-P, TI-C, TI-E, and TI-A extracts of *T. involucrata* species was tested for its hemolytic activity on human erythrocytes (O+ ve) groups.[23] The blood samples obtained from healthy volunteers was centrifuged at 10000 rpm for 5 min. 5% of erythrocyte phosphate buffer saline suspension was used for the assay. The test solution consists of various concentrations (50-200 µg/ml) of extracts, 2ml of NaCl solution (0.85%) and 5% erythrocyte suspension. The test solutions and the positive control were incubated at room temperature for 30 min. The incubated samples was centrifuged and the absorbance of the supernatant solution was measured at 540 nm. The hemolysis of red blood celss in control and test samples was calculated. Complete hemolysis was obtained in PBS buffer plus 0.1% Triton X-100 as a positive control. The percentage of hemolysis (H) was calculated from the following calculation,

% Hemolysis (H) =Ab T/Ab PC x 100

Where, "Ab T" is the absorbance for various extracts concentration, "Ab PC" is the absorbance of the positive control.

Statistical Analysis

All the experiments were performed in triplicates and the data were expressed as mean± standard deviations by using SPSS software version 16.0.

Results and Discussion

The phytochemical screening reveals the presence of various phytoconstituents mainly sterols, terpenoids and phenols in major fractions (Table 1).

In vitro Anti-arthritic activity

The *in vitro* anti-arthritic effect of *T.involucrata* leaf extracts was studied by protein denaturation method. *T.involucrata* extracts inhibitory effect on protein denaturation at different dose levels (50-200 µg/ml) is shown in Figure 1.The chloroform extract (TI-C) showed greater inhibitory activity of 93% at 200µg/mL compared to standard drug whereas; at 50 µg/ml and 100 µg/ml concentrations, it showed lower activity. However, petroleum ether (TI-P) extract exhibited consistently potent inhibitory activity at all concentrations. The TI-E and TI-A extracts showed moderate activity.

Table 1: The phytochemical screening results of leaf extracts of *T. involucrata*

Phytoconstituents	*T. involucrata* leaf extracts			
	TI-A	TI-E	TI-C	TI-P
Alkaloids	-	-	-	-
Carbohydrates	+	+	+	+
Glycosides	-	-	-	-
Phytosterols	+	+	+	+
Protein & Amino Acids	-	-	-	-
Phenols& Tannins	-	-	-	+
Terpenoids	-	+	+	+
Saponins	+	-	-	+
Flavanoids	-	-	-	-

(-) -absence, (+) - presence

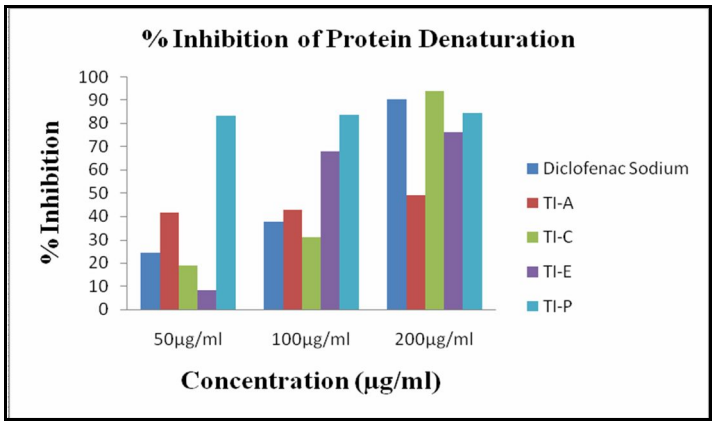

Figure 1. % Inhibition of Protein Denaturation

It is well known that the denaturation of tissue protein is one of the major causes for rheumatoid arthritis. [24] The mechanism of protein denaturation involves alteration in electrostatics, hydrogen, hydrophobic and disulphide bonding leading to the production of auto antigen. [25] Therefore, from the results it could be concluded that the chloroform (TI-C) and petroleum ether extract (TI-P) contain potent molecules with anti-arthritic property.

Hemolytic Assay

The *T.involucrata* extracts possessing potent anti-arthritic property are checked for their hemolytic property. The extracts containing biologically active molecule may not be useful if they are hemolytic in nature. The hemolytic assay on human erythrocytes of various extract concentrations of *T.involucrata* was performed. 100% hemolysis was obtained in Triton X-100. From the results obtained the chloroform (TI-C) and aqueous

(TI-A) extract showed no hemolytic property and all other extracts possessed lower hemolytic effect of less then 0.1% at all concentrations.

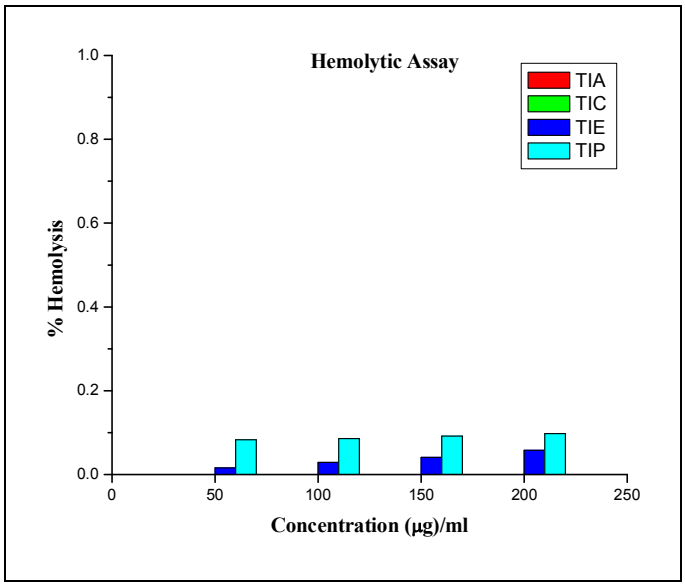

Figure 2. % Hemolysis- Hemolytic Assay

The erythrocyte model used in general for the hemolytic study because of its ready availability and its membrane similiarity with other cell membranes.[26,27] The TI-C and TI-A extract that showed no hemolytic effect suggest its non-toxic effect thus making it suitable for treatment of disease.

Conclusion

The preliminary phytochemical screening showed terpenoids, sterols, phenols and flavanoids to be the major phytoconstituents present. The *in vitro* anti-arthritic activity of *T .involucrata* leaf extracts was studied by protein denaturation method and it demonstrated that the TI-C and TI-P extract have potent inhibitory property. This activity may be due to the presence of active components such as terpenoids, sterols, phenols and flavanoids present in major. The extracts were also found to be non-hemolytic in nature and thus suitable for consumption as a drug.Thus further investigation would be carried out in isolation of the active compounds and elucidate their inhibitory mechanism in *in vivo*.

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