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RESEARCH ARTICLE

In Vitro Anti-Inflammatory and Anti-Arthritic Activity of Methanolic Extracts of *Acalypha Indica Linn., Emblica officinalis* Gaertn. and *Tridex Procumbens* L. of Gopalganj, Bihar

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Abstract

Inflammation and arthritis are commonly observed diseases among the local population of India. A phytomedicine healing these disorders could be a boon to society in the research under given. *In vitro* anti-inflammatory and anti-arthritic actions of extracts from *Acalypha indica* Linn. (Family Euphorbiaceae), *Emblica officinalis* Gaertn. (Family Euphorbiaceae) and *Tridex procumbens* Linn. (Family Asteraceae) were checked. Methanol was kept as a main solvent for extraction. In layer adjustment measure, the most inhibition in terms of percentage was seen in methanolic concentrate of *Emblica officinalis* Gaertn. (27.55%) at 300µg/ml than *Tridex procumbens* Linn. (35.55%) and it indicated huge action than the standard medication diclofenac sodium (32.73%) at 100µg/ml. So in protein denaturation test, the methanolic concentrate of roots demonstrated most extreme percentage of hindrance (58.89%) at 300µg/ml than the leaves (33.33%). The roots indicated comparable action with the standard medication diclofenac sodium (34.44%) at 100µg/ml. Also, in the egg whites test, the ethanolic concentrate of the *Acalypha indica* Linn. indicated most extreme percentage percentage of hindrance (28.62%) at 300µg/ml than roots (33.33%) and furthermore demonstrated critical movement than standard medication diclofenac sodium (23.56%) at 100µg/ml.

Keywords: Anti-Inflammatory Activity, Anti-Arthritic Activity, Membrane Stabilization, Percentage Inhibition of Protein

Introduction

Immune system of our body plays a crucial role in the entire metabolism. An overactive immune system may lead to cause certain fatal diseases because of various hypersensitive or allergic reactions which may cause numerous derangements. Loss of normal capacity to differentiate self from non-self-resulting in immune reactions against our own's cells and tissues called autoimmune diseases. Certain common autoimmune diseases like myasthenia gravis, serum sickness, perniciousanemia, reactive arthritis etc. are severe issues for medical and pharmaceutical communities because of unknown etiology [1].

According to WHO, 0.3-1% of the world population is affected from rheumatoid arthritis (RA) and among them females are three times more prone to the disease as compared to males [2].

The goal of treatment for arthritic patients are to eliminate symptoms, slow disease progression and optimize quality-of-life [3]. Therefore, before starting the treatment of rheumatoid arthritis (RA), certain goals must be kept in mind such as relief of analgesia, reduction of inflammation, protection of articular structure, maintenance of function and control of systemic involvement [4]. Presently for the treatment of rheumatoid arthritis (RA), strategies have changed from traditionally used non-steroidal anti-inflammatory drugs (NSAIDs) or disease modifying anti-rheumatic drugs (DMARDs) to novel biological agents, like Tumor Necrosis Factor (TNF) monoclonal antibody. From a long scenario Mother Nature has provided us with the benefits of pharmaceutical sciences. A wide variety of drugs and pharmaceutical formulations can be processed and compiled with the help of plant based extracts. Therefore utilization of medicinally important plant extracts through any source of application could act as an effective way to treat severe disorders.

In prior made researches, it was found that *A. indica* L. has been explored for its varied therapeutic importance but for now anti-arthritic effect of this plant has been a novel topic to explore. The leaves of *A. indica* L. can be used as an anthelmintic [5], as an emetic [6], as a laxative for rheumatoid arthritis [7], for treatment of asthma [8], diarrhea [9], syphilitic ulcer and wound healing [10] and to induce an abortion [11]. The field of therapeutics like cancer or cosmetology, *A. indica* L. has proven its benefits throughout the world.

E. officinalis Gaertn. also possesses analgesic, antipyretic and anti-cancerous activities [12]. Behind these properties, this plant shows activity for reducing cholesterol and dyslipidemia [13]. It is also operative in treatment of skin sores, wound healing and significant in skin lightening [14]. In the flash-light, *E. officinalis* Gaertn. has been also explored for its therapeutic effects and uses but ant-arthritic point of view this plant has not been revealed.

T. procumbens L. possesses anti-diabetic properties as well as imuno-enhancement [15], hepatoprotective [16] , anti-parasitic [17], anti-cancer [18], antimicrobial [19] and antihypertensive activities [20]. The study of above literature allowed the researchers to explore several medicinal andtherapeutic properties about the plant extracts of *A. indica* L. *E. officinalis* Gaertn. and *T.procumbens* L.

Materials and Methods

Sample Procurement

Collection of plant samples for preparations of plant extricates in order to assess their capability of anti-inflammation and anti-arthritic was done from the region of Hathwa, Gopalganj (Bihar). These plants are submitted in the form of herbaria (no. 117-120) in the Department of Botany, Gopehwar College, Hathwa.

In the month of December 2017 and January 2018, all three plant parts (leaves, stems and roots) were collected in the sterile bags which were autoclaved at the day of collection. Shade drying of leaves had to be performed therefore leaves were kept under shade in lab premises and it was monitored that no extra contamination was observed.

Processing of the Plant Sample

The collected samples were clean with water thoroughly to detach all the particles of soil and the stems were chopped into small pieces and then all three samples i.e., leaves, roots and stems were shade dried at room temperature for about 7-10 days. Then the dried specimens were grinded to coarse powder using a blender (applying electricity) and then filtered through a mesh to get a fine powder. After that the powdered samples were stored in sterile airtight containers.

Solvent Extraction

The powders kept for drying were weighed to 30gm and then were dissolved in 150ml of solvent of methanol separately in a soxhlet apparatus by continuous heat exposure (80-90 mV) for 48hours till the solution becomes acquit and then the extricates were transferred in different tubes. Later the extracts were concentrated in rotary vacuum evaporator at high temperature (70° C) and then the concentrated extracts were reconstituted with 10 to 15ml of methanol and the remaining extracts were stored in refrigerator for future use. For *in vitro* study, about 50g of dried powdered root, stem and leaf samples were dissolved in 250ml of methanol and extracted in soxhlet apparatus and then the extracts were stored at 4°C for future use.

In *vitro* Test Analysis

In vitro anti-inflammatory activity by HRBC membrane stabilization method

The principle involved in stabilization of human red blood cell membrane by hypotonicity induced membrane lysis [21].

Preparation of Red Blood Cell (RBCs) Suspension

Fresh whole human blood (10ml) was collected and transferred to the heparin centrifuged tubes. The tubes were centrifuged at 3000rpm for 10min and were washed three times with equal volume of normal saline. This washing process helps us to collect the pure blood cells from the whole blood. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline [22].

Heat Induced Hemolysis

The 2ml reaction mixture is consist of 1ml of test (Plant) ethanolic extract at various concentrations (100-300µg/ml) and 1ml of 10% RBCs suspension, instead of drug only saline was added to the control test tube. Diclofenac sodium was taken as a standard drug for the further comparative studies. All the centrifuged tubes containing reaction mixture were incubated in a water bath at 56°C for 30min. At the end of incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500rpm for 5min and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicate. Then the percentage of membrane stabilization activity was calculated by the formula:

Percentage inhibition = Absorbance control – Absorbance treated / Absorbance treated X 100

In vitro Anti-Arthritic Activity

Inhibition of Protein Denaturation Method

The reaction mixture (0.5ml) consisted of 0.45ml bovine serum albumin (5% aqueous solution) and 0.05ml of plant extract (ethanolic) at different concentration (0.25 mg/ml-10 mg/ml). Then the samples were incubated at 37°C for 30 minutes. After cooling of samples, 2.5ml phosphate buffer saline (pH 6.3) was added to each tube and the turbidity was measured spectrophotometrically at 660nm. For control test 0.05ml distilled water was used instead of extracts while product control test lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows:

Percentage Inhibition = Absorbance control – Absorbance treated / Absorbance treated X 100

Inhibition of Albumin Denaturation

The 5ml of reaction mixture was comprised of 0.2ml of eggs albumin (from hens egg), 2.8ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentrations of methanolic extracts and similar volume of double distilled water was served as control. Then the mixture was incubated at 37°C in BOD incubator for about 15mins and then heated at 70°C for 5mins. After cooling, their absorbance was measured at 660nm by using pure blank. Diclofenac sodium (Standard drug) was used as reference drug and treated as such for determination of absorbance. The percentage inhibition of protein denaturation was calculated as follows:

Percentage Inhibition = Absorbance control – Absorbance treated / Absorbance treated X 100

Results

The plant extracts of *A. indica* L., *E. officinalis* Gaertn. and *T. procumbens* L. were collected and the leaves, stems and roots were shade dried and grinded. The powdered sample was then extracted by using soxhlet apparatus with methanol.

***In vitro* Anti-inflammatory Activity by HRBC Membrane Stabilization Method**

Anti-inflammatory action of *A. indica* L. (Leaves, Stems and Roots)			
Concentration (ppm)	Leaves	Roots	Stems
100	17.00%	21.80%	13.40%
200	20.00%	29.00%	16.00%
300	22.80%	32.20%	18.90%
400	23.70%	38.20%	19.00%
500	25.00%	39.00%	22.00%

Table 1: Anti-inflammatory action of *A. indica* L. by HRBC membrane stabilization method

Anti-inflammatory action of *E. officinalis* Gaertn. (Leaves, Stems and Roots)			
Concentration (ppm)	Leaves	Roots	Stems
100	14.70%	16.80%	13.40%
200	26.20%	21.24%	15.00%
300	28.30%	23.20%	17.90%
400	29.42%	27.20%	18.00%
500	32.00%	29.00%	19.20%

Table 2: Anti-inflammatory action of *E. officinalis* Gaertn. by HRBC membrane stabilization method

Anti-Inflammatory action of *T. procumbens* L. (Leaves, Stems and Roots)			
Concentration (ppm)	Leaves	Roots	Stems
100	18.70%	14.80%	14.40%
200	28.20%	23.24%	17.00%
300	29.30%	27.20%	18.90%
400	32.42%	29.20%	19.50%
500	34.00%	31.33%	20.26%

Table 3: Anti-inflammatory action of *T. procumbens* L. by HRBC Membrane Stabilization Method

The maximum percentage stabilization was observed in the DMSO and ethanolic extract of leaves. *T.procumbens* L. observed to be 32.42% at 400µg/ml (Table 3) as compared to leaves of *E. officinalis* Gaertn. which showed 27.2 % at 300 µg/ml. The standard applied in this experiment was diclofenac sodium. The root extract of the *A. indica* L. gave 38.2% of 400µg/ml. Similar work was accessed by [23] where 27.4% percentage inhibition was observed in extracts of *C. asiatica* L. in roots which was comparable to the values of *E. officinalis* Gaertn. in the present study.

In *vitro* Anti-arthritic activity by inhibition of Protein Denaturation Method

The effects of ethanolic, methanolic and DMSO extract on inhibition of protein denaturation are shown in Figures 3 and 4. Extracts from leaves, stems and roots at different dose congregation assigned significant protections against degradations of proteins. The maximum percentage inhibition was observed in ethanolic extract of roots about 58.89% at 300µg/ml as compared to leaves 53.33%. It possesses significant activity comparable to that of diclofenac sodium (100µg/ml).

From the results of present study it can be stated that ethanolic extracts of leaves and roots of *A. indica* L., *E. officinalis* Gaertn. and *T. procumbens* L., are capable of controlling the production of auto antigens and inhibit denaturation of protein in rheumatic disease.

The present study revealed that in vitro anti-inflammatory activity of three plants extracts on HRBC membrane stabilization method where the ethanolic extract of leaves of *A. indica* L., *E. officinalis* Gaertn. and *T. procumbens* L. produced maximum percentage of stabilization as 36% at 300µl than that of roots and produce significant percentage of stabilization compared to that of standard diclofenac sodium as mentioned in Table 4 and Table 5. It reveals that the leaves of *E. officinalis* Gaertn. contained anti-inflammatory activity whereas there are previous reports repeated in this plant, but [21] reported similar result in extracts of leaves of *Rhizopora mucronata* Lam. With significant percentage of stabilization about 95% at 400 mg/ml than that of standard diclofenac sodium (37%) by [11] reported the significant percentage of stabilization as 29.90%, 35.90% and 24.20% in the methanol, ethanol and aqueous extract of *Physalis angulate* L. compared to diclofenac sodium (29.80%) and represented in the order of ethanol>water>methanol and also the methanolic extract of leaves of *Cocculus hirsutus* L. showed maximum percentage stabilization of about 88.8% at 1000µg/ml than that of other *in vivo* and *in vitro* (callus) parts was reported [24] reported that the methanolic extract of *Myxopyrum serratulum* A.W.Hill. showed percentage stabilization as 27.25% at 200µg/ml compared to standard diclofenac sodium.

Further in plant extracts anti-arthritic activity were determined by tests *In vitro* ant- arthritic activity by inhibition of albumin denaturation method. The values observed for *T. procumbens* Linn. Was more accurately comparative with diclofenac sodium. The research was also carried out for anti-arthritic activity of these plant extracts. The studies represented a common drop down of arthritic and inflammation by the plant extracts. The results of anti-arthritic activity have been demonstrated in the figure mentioned below.

Anti-Arthritic activity of *Acalypha indica* L.			
Concentration (ppm)	Leaves	Stems	Roots
100	23.00%	25.00%	26.00%
200	25.00%	28.00%	28.00%
300	27.00%	32.00%	29.00%
400	32.00%	34.00%	31.00%
500	36.00%	37.00%	35.00%

Table 4: Anti-arthritic activity of *Acalypha indica* L.

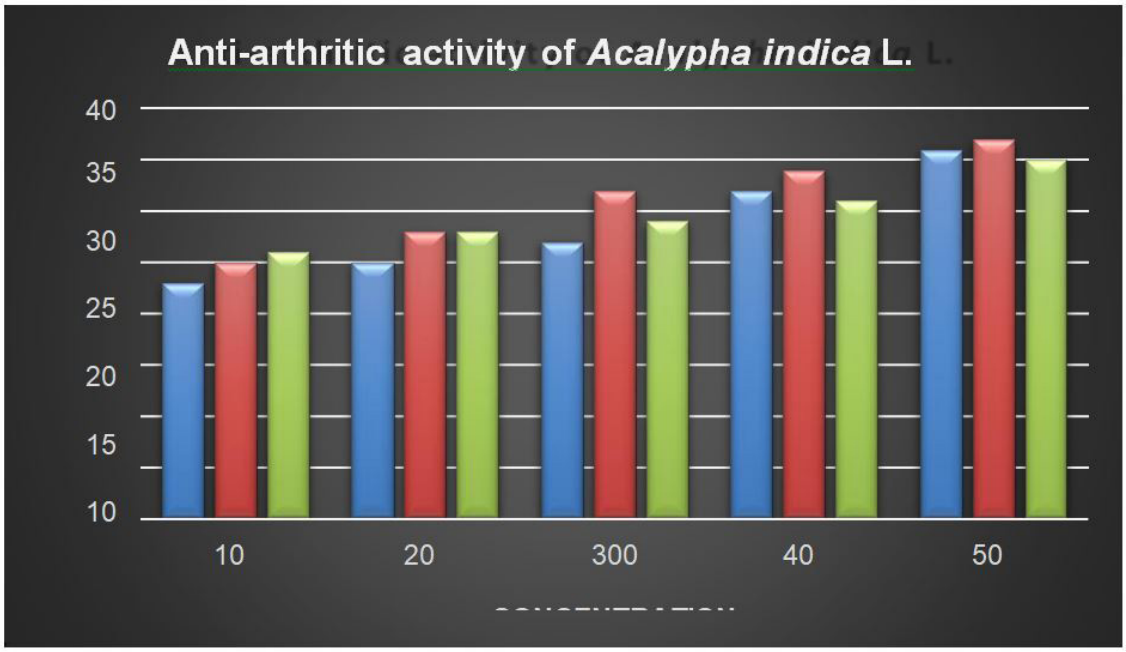

Figure 1: Anti-arthritic activity of *A. indica* L

Anti-arthritic activity of *E. officinalis* L.			
Concentration (ppm)	Leaves	Stems	Roots
100	21.00%	21.20%	26.00%
200	24.00%	24.32%	25.00%
300	27.00%	26.28%	27.00%
400	34.00%	29.12%	32.00%
500	36.00%	34.00%	37.00%

Table 5: Anti-arthritic activity of *E. officinalis* L.

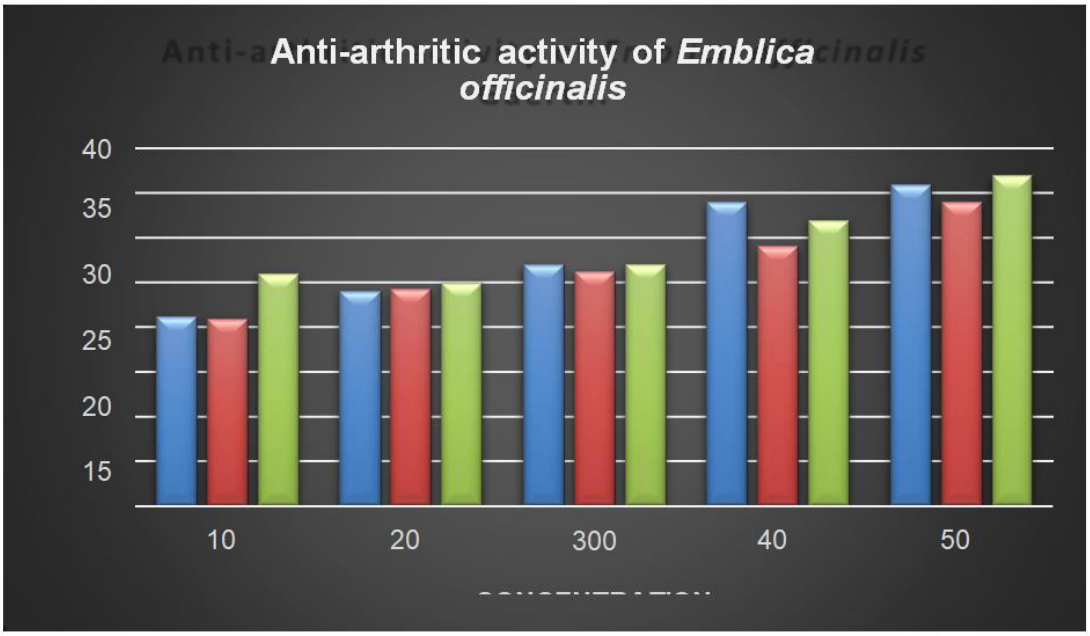

Figure 2: Anti-arthritic activity of *E. officinalis* L.

Anti-arthritic activity of *T. procumbens* L.			
Concentration (ppm)	Leaves	Stems	Roots
100	27.00%	17.00%	26.00%
200	29.00%	19.00%	29.00%
300	41.00%	27.00%	32.00%
400	42.00%	32.00%	37.00%
500	47.00%	38.00%	39.00%

Table 6: Anti-arthritic activity of *T. procumbens* L.

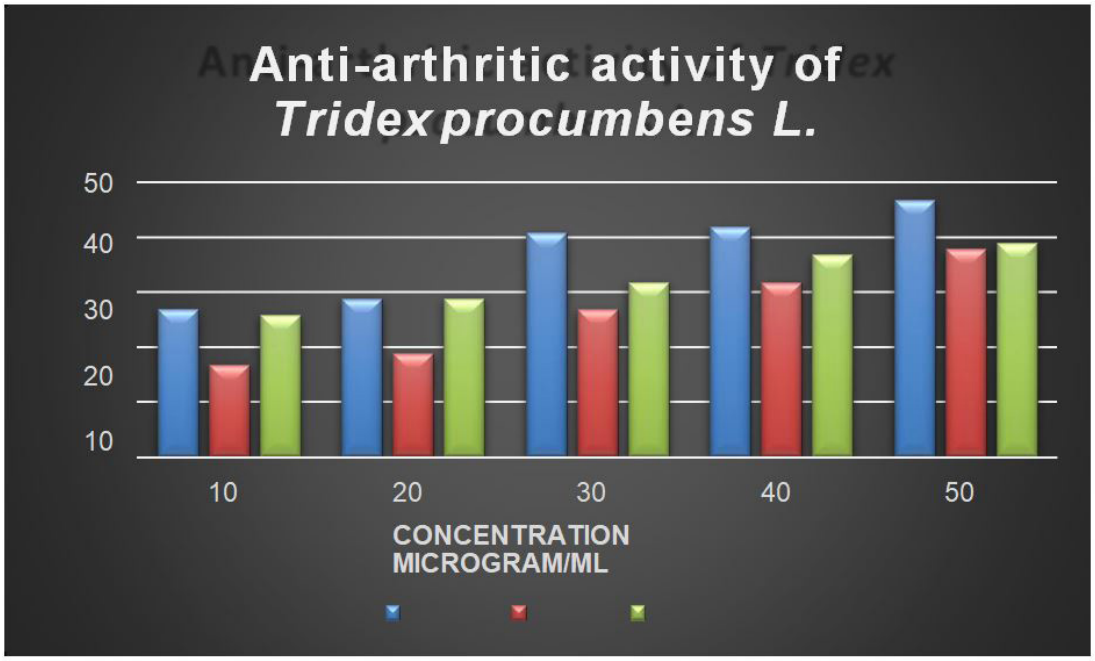

Figure 3: Anti-arthritic activity of *T. procumbens* L.

In light of the present research work, the proximity of terpenoids, tannins, steroids and polyphenol mixers have been observed to be effective for the action of anti-inflammatory and anti-arthritic effects. Yet a similar report was accounted for the concentrates of leaves of *Rhizopora mucronata* Lam. which demonstrated most extreme level of restraint and greatest level of hindrance in egg whites. Denaturation measure was accounted by [21] detailed the critical level of restraint of protein denaturation by the methanolic concentrate of leaves of *Proteum serratum* Wall ex. Colebr. The ethanol, methanol and fluid concentrate of *Physalis angulate* L. produce noteworthy level of restraint of protein [1] .worked in detail the greatest level of hindrance of protein denaturation in the methanolic concentrate of the leaves of *Cocculus hirsutus* L.

In the above research it was fully observed that whether these plants have been used in traditional medicines since decades but have not been explored much for their anti-inflammatory and anti-arthritic effects, though the traditional knowledge focuses that these plants have been ordinarily or sub-ordinarily used in several purposes. In one assay the results were compared with acetyl salicylic acid and in other case it was compared with diclofenac sodium.

This research also targeted to see the better response of the extract whether made with ethanol, methanol or DMSO has been found efficient in all the cases of study *T. procumbens* L. with 56% of anti-inflammatory property has been found effective and of use.

Conclusion

The result of the study shows that the ethanolic extract of the leaves of all the plants investigated can be used in the treatment of inflammation and arthritis due to the significant percentage of membrane stabilization and inhibition of protein denaturation. A phytomedicine, healing these disorders could be a boon to society in the research under given *in vitro* anti-inflammatory and anti-arthritic actions of extracts from *A. indica* L, *E. officinalis* Gaertn. and *T.procumbens* L. were checked. Methanol was kept as a main solvent for extraction. In layer adjustment measure, the most inhibition in terms of percentage was seen in methanolic concentrates of *E. officinalis* Gaertn. than *T. procumbents* L. and it indicated huge action than the standard medication diclofenac sodium. So also, in the protein denaturation test, the methanolic concentrate of roots demonstrated most extreme percentage of hindrance (38.89%) at 300µg/ml than the leaves. The roots indicated the comparable action with the standard medication diclofenac sodium at 100µg/ml. Also, in the egg whites denaturation test, the ethanolic concentrate of *A. indica* L. has indicated most extreme percentage of hindrance (38.62%) than roots and furthermore demonstrated critical movement than standard medication diclofenac sodium. The utilization of herbal medicinal plants in the treatment of inflammation and arthritis could be a boon to society because chemical methods came with higher number of side effects.

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