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In-vitro anti-inflammatory activity of different extracts of flowers of *Tridex procumbens*

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ABSTRACT

AIM- The main of study is to evaluate the *In-vitro* anti-inflammatory activity of different extracts of flowers of *Tridex procumbens.* **Material & Methods-**Successive solvent extraction method was followed for extraction by using different solvents i.e. petroleum ether, chloroform, methanol, butanol and water. Preliminary phytochemical screening method was performed for the presence of different phytoconstituents. For the evaluation of anti-inflammatory activity, protein denaturation and proteinase inhibitory method were performed. Diclofenac sodium was used as standard. **Result-** Among all the extracts, methanolic extract of *Tridex procumbens* showed 80.23% inhibition. The petroleum ether, chloroform, n-butanolic and water extract had shown the 12.23, 23.33, 20.45 and 36.20% inhibition respectively at 500 µg/ml concentration. The Diclofenac sodium showed 83.28 % inhibition against denaturation of protein. The different extracts were evaluated by using this method. Methanolic extract of *Tridex procumbens* showed 74.50% inhibition at 500 µg/ml. The petroleum ether, chloroform, n-butanolic and water extracts had shown the 30.16%, 41.22%, 43.28% and 31.97% inhibition respectively at 500 µg/ml. The Diclofenac sodium showed 79.21% inhibition against proteinase inhibitory activity at 100 µg/ml. **Conclusion-** Besides from the obvious therapeutic importance, methanolic extract would be useful in understanding the mechanism of diseases with higher levels of cellular and molecular level. These components could serve as lead molecules for development of prospective anti-arthritic agents. Further detailed studies are required to isolate the active phytoconstituents from methanolic extract which is responsible for anti-arthritic activity.

Keywords: Protein denaturation model, proteinase inhibitory method, methanolic extract, anti-inflammatory activity, *Tridex procumbens*

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INTRODUCTION

Inflammation is usual and essential protective response to the harmful stimuli such as infectious agents, antigen-antibody reactions, thermal, chemical, physical agents, and ischemia (Goldyne *et al.,* 1984). It is caused by a assortment of stimuli, including physical damage, UV irradiation, microbial attack, and immune reactions. The classical facial appearance of inflammation is redness, warmth, swelling, and pain. Inflammation cascades can lead to the expansion of diseases such as chronic asthma, arthritis, multiple sclerosis, inflammatory bowel disease, and psoriasis. Many of these diseases are incapacitating and are flattering increasingly common in our ageing society[1].

Immunosuppressive DMARDs to produce some or the other side effects connected with suppression of immunity. On prolonged use, these drugs can cause renal complications, renal insufficiency, and hyperkalemia particularly in CHF, cirrhotic and ascetic at higher doses in patients[2,3].

Traditionally herbal plants were used equally outwardly and inside for treating inflammatory circumstances such as arthritis. The confident persuade of herbal medicine in alternating pathophysiology of arthritis has resulted in a substantial increase in their use as a treatment for arthritis[4]. In the absence of any scientific confirmation for anti-arthritic activity in inflammatory conditions of *Tridex procumbens*, there is a need in scientifically establishing the anti arthritic activity of different bioactive extracts with fewer side effects in assessment with existing synthetic drugs.

MATERIAL & METHODS

Plant Materials

The flowers of *Tridex procumbens* were collected from the college premises and from nursery.

Authentification of Plant Materials

Flowers of plant were taxonomically identified by Dr. Anurag Titov, Professor, Department of Botany, Govt. Madhav Sciences, PG College, Dewas Road, Ujjain and herbarium specimen was submitted in Department of Botany for future references.

Methods

Preparation of Total Crude Extract

Flowers of plant were dried under shade and subjected to coarse powder for extraction process. Accurately weighed (200 gm) quantity of flowers of *Tridex procumbens* were extracted using petroleum ether, chloroform, methanol, butanol and finally water by soxhlet apparatus for 72 hr. The all extracts were dried under the reduced pressure to get crude extracts. After drying, the respective extracts were weighed and percent yield was determined[5].

Preliminary Phytochemical Tests

Qualitative chemical tests of all extracts were subjected to various chemical tests to detect various presences of various phytoconstituents[5,6].

Evaluation of *in-vitro* anti-arthritic activity

Inhibition of protein denaturation method:

All the extracts (pet ether, chloroform, methanol, butanol and water) of *Tridex procumbens* were evaluated by this method. The following procedure was followed for evaluating the percent of inhibition of protein denaturation.

Control solution (50 ml) consists of 2ml of Egg albumin (from fresh hen's egg) and 28 ml of phosphate buffer (PBS, pH 6.4) and 20ml distilled water.

Standard drug (50 ml) consists of 2ml of Egg albumin and 28 ml of phosphate buffer and 20 ml of various concentrations of standard drug Diclofenac sodium (20, 40, 60, 80 & 100 µg/ml).

Test solution (50 ml) consists of 2ml of Egg albumin and 28 ml of phosphate buffer and 20 ml of various concentrations of petroleum ether, chloroform, methanol, n-butanol & aqueous extracts (100, 200, 300, 400, 500 µg/ml).

All of the above reaction mixtures were adjusted to pH 6.4, using a small amount of 1N HCl. The samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling, the absorbance of the above solutions was measured using UV- spectrophotometer at 660 nm. The percent inhibition of protein denaturation was calculated using the following formula[7,8].

Percent inhibition = (Vt/Vc-1) x 100)

Where, Vt= absorbance of test sample, Vc= absorbance of control

Proteinase inhibitory activity

Different extracts of *Tridex procumbens* were evaluated by this model. The following procedure was followed for evaluating the Proteinase Inhibitory Activity.

Control solution (50 ml) 20 ml consists of 0.06 mg Trypsin, 10 ml 20mM Tris HCl buffer (pH 7.4) and 10 ml distilled water.

Standard drug (50 ml) 20 ml consists of 0.06 mg Trypsin, 10 ml 20mM Tris HCl buffer (pH 7.4) and 10 ml of various concentrations of standard drug Diclofenac sodium. (20, 40, 60, 80 & 100 µg/ml).

Test solution (50 ml) 20 ml consists of 0.06 mg Trypsin, 10 ml 20mM Tris HCl buffer (pH 7.4) and 10 ml of various concentrations of petroleum ether, chloroform, methanol, n-butanol & aqueous extracts, obtained from *Tridex procumbens* (100, 200, 300, 400, 500 µg/ml).

All the reactions were incubated at 37° C for 5 minutes and then 10 ml of 0.8% (w/v) casein was added. Again the samples were incubated for an additional 20 min and 10 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed as triplicate. The percent inhibition of proteinase inhibitory activity was calculated[9].

Percent inhibition =

(Abs Control – Abs Sample) x 100 / Abs control.

RESULTS

Extractive Value Determination

Dried flowers of *Tridex procumbens* were extracted using pet ether, chloroform, methanol, butanol and finally water by soxhlet apparatus. The percent yields were determined by using the following formula.

$$\text{Percent yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Table 1: Different extracts with their appearance and % yield (in gm)

S. No.	Extracts	Color of dried extracts	Consistency of dried extracts	% Yield (W/W)
1	Pet ether extracts of *Tridex procumbens*	Dark brown	Sticky	8.52 %
2	Chloroform extracts of *Tridex procumbens*	Dark Green	Sticky	6.56 %
3	Methanolic extracts of *Tridex procumbens*	Dark Yellowish Green	Sticky	12.23 %
4	Butanolic extracts of *Tridex procumbens*	Dark Yellowish Green	Sticky	4.88%
5	Aqueous extracts of *Tridex procumbens*	Dark Green	Sticky	5.58 %

Preliminary Phytochemical Screening

The preliminary phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolics compounds.

Evaluation of *in-vitro* anti-arthritic activity of *Tridex procumbens*

Protein Denaturation Methods

Among all the extracts, methanolic extract of *Tridex procumbens* showed 80.23% inhibition. The petroleum ether, chloroform, n-butanolic and water extract had shown the 12.23, 23.33, 20.45 and 36.20% inhibition respectively at 500 µg/ml concentration. The Diclofenac sodium showed 83.28 % inhibition against denaturation of protein. The results are summarized in Table No. 2.

Table 2: Effect of different extracts on Protein Denaturation Method.

S. No.	Treatment	Concentration µg/ml	% Inhibition
1	Control	----	-----
2	Petroleum ether Extract	100	8.61
		200	9.44
		300	10.42
		400	11.41
		500	**12.23**
3	Chloroform Extract	100	12.55
		200	13.82
		300	14.24
		400	20.76
		500	**23.33**
4	Methanolic Extract	100	30.72
		200	37.41
		300	42.58
		400	60.99
		500	**80.23**
5	n-butanolic Extract	100	10.33
		200	12.42
		300	15.57
		400	18.59
		500	**20.45**
6	Aqueous Extract	100	13.73
		200	18.22
		300	20.33
		400	30.45
		500	**36.20**
7	Diclofenac Sodium	20	33.44
		40	36.55
		60	41.56
		80	70.88
		100	**83.28**

Proteinase inhibitory action:

The different extracts were evaluated by using this method. Methanolic extract of *Tridex procumbens* showed 74.50% inhibition at 500 µg/ml. The petroleum ether, chloroform, n-butanolic and water extracts had shown the 30.16%, 41.22%, 43.28% and 31.97% inhibition respectively at 500 µg/ml. The Diclofenac sodium showed 79.21% inhibition against proteinase inhibitory activity at 100 µg/ml. The results are summarized in Table No 3.

Table 3: Effect of different extracts on proteinase inhibitory activity

S. No.	Treatment	Concentration µg/ml	% Inhibition
1	Control	----	-----
2	Petroleum ether Extract	100	17.56
		200	21.31
		300	27.34
		400	28.22
		500	**30.16**
3	Chloroform Extract	100	24.51
		200	35.55
		300	38.76

		400	40.31
		500	**41.22**
4	Methanolic Extract	100	35.64
		200	39.88
		300	47.98
		400	59.64
		500	**74.50**
5	n-butanolic Extract	100	32.22
		200	36.46
		300	37.88
		400	40.89
		500	**43.28**
6	Aqueous Fraction	100	17.54
		200	21.29
		300	25.78
		400	30.58
		500	**31.97**
7	Diclofenac Sodium	20	33.82
		40	35.44
		60	44.55
		80	60.51
		100	**79.21**

DISCUSSION

Rheumatoid arthritis is a unrelieved inflammatory disease which leads to the smash up of synovial membranes, cartilage and bone. Although etiology and pathogenesis of RA is poorly unspoken, pro-inflammatory cytokines are well thought-out to be one of the most imperative peacekeepers involved in the pathogenesis of RA (Mclnnes & Schett, 2007). Various cytokines i.e. Tumor necrosis factor (TNF-α) & Interleukin-1 (IL-1) is known to contribute a critical role in the pathogenic mechanisms of arthritis and is well recognized as another pro-inflammatory cytokine concerned in arthritis[10].

The unbelievable development in the area of synthetic drugs throughout present era is accompanied by various undesirable side effects. Whereas plants still hold their own unique breathing space, with lesser side effects[11].

Thus, in the present investigation, an effort was made to evaluate the anti-arthritic activity of *Tridex procumbens* on the basis of ayurveda and their traditional uses in a suitable experimental animal.

In the preliminary study, dried powders of flowers plant was extracted by using pet ether, chloroform, methanol, butanol and finally water. The extracts were dried and screened for the presence of various active constituents. The extracts showed the presence of alkaloids, terpenoids, flavonoids, glycosides, phenolic compounds, tannins, steroids and fatty acids. For the beginning assessment, all extracts were evaluated by *in-vitro* anti-arthritic activity i.e. protein denaturation inhibitory activity & proteinase inhibitory activity.

The arthritic disease sequence was correlated with the weakness of the lysosomal membranes, denaturation of proteins & discharge of inflammatory mediators. Denaturation of proteins is a well known reason of inflammation. The preponderance of biological proteins misplaces their biological functions when denatured. Within body, denaturation of proteins causes production of auto-antigens in arthritic disorders[12,13]. Agents that can put off protein denaturation consequently would be meaningful for anti-arthritic activity.

Serine proteinase from inflammatory cells is worried in various inflammatory disorders such as arthritis and pulmonary emphysema. Neutrophils are known to be a wealthy starting place of serine proteinase and are confined to a small area at Lysosomes. Deficiency of protease inhibitors in transmission is the major risk factor for enlargement of inflammatory disorder[14,15,16].

In our examination, the different extracts were studied for protein denaturation as well as proteinase inhibitory activity. The methanolic extract was found to be effectual in inhibiting heat induced albumin denaturation & proteinase inhibition at different concentrations.

CONCLUSION

This study was designed to find out and prove the claims put forth by the traditional and folk medicine of applicability of *Tridex procumbens* that have been used in crippling arthritis and frozen joints in Siddha and Ayurvedic system of medicine.

Besides from the obvious therapeutic importance, methanolic extract would be useful in understanding the mechanism of diseases with higher levels of cellular and molecular level. These components could serve as lead molecules for development of prospective anti-arthritic agents. Further detailed studies are required to isolate the active phytoconstituents from methanolic extract which is responsible for anti-arthritic activity.

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