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IN-VITRO ANTI-INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITY OF N-BUTANOL FRACTION FROM *TELOSMA PALLIDA* (ROXB) CRAIB LEAF

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ABSTRACT

Telosma pallida (Roxb) Craib (TP) is a perennial climber found prominently in the region Saurashtra. Traditionally it is described to neutralize *tridosha* (*vata*, *pitta*, *kapha*). The plant is fiber-rich and edible in this region. An *In-vitro* anti-inflammatory activity was evaluated for an n-butanol fraction of the TP leaf by two methods i.e. inhibition of albumin denaturation, Red Blood Cell (RBC) membrane stabilization method and. An n-butanol fraction (nBF) was found to show triterpene saponin in the qualitative phytochemical screening. In the present study, nBF fraction has produced inhibition up to 50.71 ± 0.36% in case of RBC membrane lysis method with IC_{50} value 931.18 ± 3.82 (p<0.001), while 53.03 ± 1.06% inhibition with IC_{50} value 928.08 ± 20.50 (p<0.001) was found in the case of anti-protein denaturation. Study first time demonstrated the in-vitro anti-inflammatory and anti-arthritic potential of the *T. pallida* leaf.

KEYWORDS: *Pergularia pallida*; anti-arthritic activity; denaturation; inflammation; HRBC.

INTRODUCTION

Telosma pallida (Roxb) Craib is also known as *Pergularia pallida*. *T. pallida* is a weed found throughout the surroundings of Junagadh region of India. Traditionally, leaves and flowers of this plant are commonly used as a vegetable in this area. Flowers are more prominent in the monsoon season. The flowers of the *T. pallida* are seasonal. The flowering season is July to

September i.e. during monsoon. Flower is found to be yellowish-green, aromatic, and having five petals. Flowers are the comestible vegetable in the Saurashtra region of India.[1]

The aerial part of the *T. pallida* was found to have sarcogenin, a pregnane derivative and pregnane ester glycosides.[2, 3]

The root of the plant has been reported for the presence of phenanthroindolizidine alkaloids namely pergularinine, tylophoridine etc.[4] These alkaloids have dihydrofolate reductase and cell growth inhibitor activity in-vitro.[5] These alkaloids have also proved their antitumor potential by inhibiting enzyme thymidylate synthase enzyme.[6]

The root of this plant has been used traditionally as galactagogue in the various forest areas of the Andhra Pradesh, India. This indigenous practice has been carried out by crushing the root with long pepper and given with jiggery.[7]

The Latex from the fruit of *T. pallida* has been used as a traditional treatment of leucoderma by the *Kol* tribes of Vindhyan region of Uttar Pradesh, India. The fresh latex is applied to the affected part of the skin and after continues practice some improvements have been observed.[8]

Flowers are reported to have various nutrients like minerals, vitamin, protein, fibers etc. Phytochemical screening revealed the presence of flavonones and chlorogenic acid.[9]

In the present study, we explored the in-vitro anti-inflammatory potential of *T. pallida* leaf fraction the first time.

MATERIALS AND METHODS

Collection and authentication of plant material

Plant material was collected each year during the July to September months of 2012 to 2015 from Joshipura area (21.55711ºN, 70.44772ºE) of Junagadh district, Gujarat, India. Plant specimen was identified by Dr H. B. Singh, Chief Scientist and Head of Department of Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources, New Delhi, India. A voucher specimen (SSPC/DPC/VS/01) was submitted in the Department of Pharmacognosy, Shree Swaminarayan Pharmacy College, Kevadia Colony, Dist. Narmada, Gujarat, India, for future reference.

Extraction and phytochemical screening

Leaves were dried well in shade for 15 days. Then fine powder was made and stored in an air-tight container until use.

500 g of powder was extracted with 2.5 L of Petroleum ether twice to remove the fat and chlorophyll. Then it was re-extracted with 2.5 L methanol for three times. The methanol extract was combined and methanol was evaporated by distillation. The crude methanol extract was dried over anhydrous sodium sulfate for 3 days. Then again, methanol extract was suspended in 200 mL of distilled water & shake with chloroform, ethyl acetate, and n-butanol (pre-saturated with water by mixing 1:1 ratio of distilled water and n-butanol in separating funnel for 3 hours). An n-butanol fraction was separated and dried. A light brownish powdered mass was obtained.

Qualitative phytochemical screening of nBF showed the presence of triterpenes and steroidal compounds.[10]

RBC membrane stabilization method

The basic principle behind this method is stabilization of RBC membrane lysis induced by hypotonicity as mentioned by Gandhisan[11] and Sadique.[12]

The collected blood (from local slaughter house, Junagadh, Gujarat) was mixed with equal volume of alsever's solution (sterilized by autoclave). The composition of Alsever solution was as follow; 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water. The mixture was centrifuged for 10 min at 3000 rpm and then washed with isosaline (0.85%, pH 7.2).

A Reaction mixture which contained 0.5 mL of various concentrations (25, 50, 100, 200, 500, 1000 µg/mL in phosphate buffer pH 7.4) of nBF and Diclofenac sodium as standard in the range of 25, 50, 100 µg/mL, 1 mL phosphate buffer, 2 mL of hyposaline (0.36%), 0.5 mL of RBC suspension, were incubated at 37°C for 30 min and centrifuged for 20 min at 3000 rpm. The hemoglobin content in the suspension was determined using a spectrophotometer at 560 nm. Total hemolysis of blood in the distilled water was considered as 100% and used as a control. The HRBC membrane stabilization or protection was calculated using the formula;

% stabilization $= 100-[(A_1$ (sample/standard) / A_0 (Control)] $\times$ 100, where A1 was absorbance of sample or standard and A0 was absorbance of control.

Inhibition of albumin denaturation

An inhibition of albumin denaturation was performed as per method given by Mizushima and Kobayashi[13] with minor modification.

Reaction mixture contained sample solution in various concentrations of nBF (25, 50, 100, 200, 500, 1000 µg/mL), 1% solution of bovine albumin fraction V, pH of the reaction mixture set at 6.3 using 1N HCl. The samples were incubated at 37°C for 20 min and then heated to 51°C for 20 min. After cooling the turbidity was measured spectrophotometrically at 660 nm. The results were compared to Diclofenac sodium (25,50,100 µg/ml). Percent inhibition of protein denaturation was calculated by using the same above formula.

Statistical analysis

All the experiments were performed three times and expressed as mean ± standard deviation (SD). IC50 value (µg/mL) effective concentration at which half maximum activity is produced was calculated for each assay. Statistical comparisons were calculated by student's *t*-test. The difference was counted highly significant at *p*-value< 0.01.

RESULT AND DISCUSSION

In the present study, an n-butanol fraction of *T. pallida* leaf for in-vitro anti-inflammatory activity first time by the two commonly used methods.

Inflammation is the primary physiological response to the external or internal stimuli. In other words, it's like a defense mechanism.[14]

Protein denaturation is one of the primary causes of the inflammatory and arthritic diseases. Now, this mechanism is well studied and well documented.[15]

During the inflammatory conditions, lysosomal enzymes released and produce a variety of disorders. Acute and chronic inflammation condition can be correlated with the extracellular activity of these enzymes.[16, 17]

As shown in table 1, the n-butanol fraction of *T. pallida* leaf is capable of producing inhibition of HRBC membrane lysis in a dose-dependent manner. This activity is comparable to the standard anti-inflammatory drug, Diclofenac sodium from 50 to 1000 µg/mL. Maximum inhibition was found to be 50.71 ± 0.36% in case of HRBC membrane lysis inhibition.

Anti-protein denaturation can be dealt with anti-arthritic activity. As in arthritis, a number of autoantigens is produced due to denaturation of the protein present in the body.[18,19] As shown in table 1, the crude n-butanol fraction can produce up to 53.03 ±1.06% anti-denaturation as compared to 50µg/ml concentration of standard drug (diclofenac sodium). Though, IC_{50} values in both the cases are slightly higher which indicates the mild to moderate anti-inflammatory activity but pure phytochemical from the fraction may produce more strong activity as compared to crude one.

The pharmacological activity of n-butanol fraction may be due to the presence of various phytochemicals like triterpene or other related glycosides. In the conclusion, anti-inflammatory activity of the n-butanol fraction of *T. pallida* leaf may be deduced protecting the tissues from various autoantigens or preventing the extracellular influx of lysosomal enzymes. The use of plant or specific extract may be used in the phytotherapy of various inflammatory diseases. Further work is going on to detect the presence of various phytochemicals.

Table 1 Effect of *T. pallida* n-butanol extract on HRBC membrane lysis and protein denaturation (inhibition %)

HRBC membrane lysis (inhibition %)			**Anti-protein denaturation (%)**		
Concentration (µg/mL)	***T. pallida* % Inhibition**	**Diclofenac sodium inhibition**	**Concentration (µg/mL)**	***T. pallida* % Inhibition**	**Diclofenac Sodium inhibition**
25	9.87 ± 0.12	18.51 ± 0.83	25	11.35 ± 0.15	45.70 ± 0.16
50	19.60 ± 0.26	37.44 ± 0.88	50	15.49 ± 0.34	53.45 ± 0.18
100	26.43 ± 0.24	64.00 ± 2.5	100	22.29 ± 0.21	75.29 ± 1.96
200	30.95 ± 0.23		200	25.97 ± 0.28	
500	37.33 ± 0.38		500	32.40 ± 0.25	
1000	50.71 ± 0.36		1000	53.03 ± 1.06	
IC_{50}	931.18 ± 3.82***	75.36 ± 2.12	**IC_{50}**	928.08 ± 20.50***	38.06 ± 0.14

Each value is the mean ± SD (n=3), ***p<0.01 (student's *t*-test unpaired) compared with corresponding control.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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