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Short Communication

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In-vitro anti-inflammatory and anti-arthritic activities of ethyl acetate extract of *Skimmia anquetilia* leaves

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ABSTRACT

Current investigations were carried out for the validation of *in-vitro* anti-inflammatory and anti-arthritic property of leaves of *Skimmia anquetilia* using red blood cells membrane stabilization and protein denaturation methods respectively. Defatted ethylacetate extracts at different concentration levels (50, 100, 200 and 400 mg/ml) were used in these studies. Dose dependent inhibition of protein denaturation was found 92.41% at 400 mg/ml of extracts and 96.21 % at 100 mg/ml of acetyl salicylic acid as standard in antiarthritic study. Similarly, in membrane stabilization methods, maximum effect found 90.70 % at 400 mg/ml of extracts and 94.88 % at 100 mg/ml of diclofenac sodium as standard for anti-inflammatory evaluation. The results concluded that, ethyl acetate extract of *S. anquetilia* leaves has shown significant ($^{*a}P<0.05$) anti-inflammatory and anti-arthritic effects.

KEYWORDS: *Skimmia anquetilia*, *In-vitro* anti-inflammatory, Anti-arthritic activity, Membrane stabilization assay, Protein denaturation method

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INTRODUCTION

Inflammation is a self defense mechanism of the body to protect against reaction to infection, irritation or allegens or any other harmful irritation. It is a part of the host defense mechanisms. It is known to be involved in the inflammatory reactions such as release of histamine, bradykinin, prostaglandins, extravasations, cell migration, tissue breakdown and repair which are aimed at host defense and usually activated in most disease condition. The critical role of inappropriate inflammation is becoming accepted in many diseases that affect man, including cardiovascular diseases, inflammatory and autoimmune disorders, neurodegenerative conditions, infection and cancer[1]. Stabilization of lysosomal membrane is important in inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil for example bactericidal enzymes and proteases, which cause tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane[2]. HRBC or erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of human red blood cell membrane by hypotonicity induced membrane lysis can be taken as an *in-vitro* measure of anti-inflammatory activity of the untapped drugs or plant extracts. *Skimmia anquetilia* is an aromatic gregarious shrub belonging to family Rutaceae. It is mostly found in Western part of Himalayas and Kashmir in India. Traditionally, the leaf infusion of *S. anquetilia* is taken for treatment of headache, freshness and general fever[1,3]. The leaves of *S. anquetilia* are aromatic and known to contain linalool, geraniol, pinene, scopoletin, skimmianine, umbelliferone[3]. The present investigations are scientifically validated *in vitro* anti- inflammatory effects using human red blood cell membrane stabilization and *anti*-arthritic activity by protein denaturation assay methods.

MATERIALS AND METHODS

Plant Material

Skimmia anquetilia leaves were collected from Gulmarg area of Kashmir (J&K, India). The defatted ethyl acetate extracts of *S. anquetilia* leaves (EESA) were prepared from air dried leaves.

In vitro Anti-inflammatory Activity

Red blood cell membrane stabilization method

Human red blood cell (HRBC) membrane stabilization method was performed as described by Kumar *et al*[4]. The blood was

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collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3 000 rpm. The packed cells were washed with isosaline and a 10% suspension was made.

Ehyl acetate extracts of *S. anquetilia* leaves was prepared and evaluated at different levels 100, 200 and 400 mg/mL) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. It was incubated at 37^{0}C for 30 min and centrifuged at 3 000 rpm for 20 min. The hemoglobin content of the supernatant solution was estimated with UV spectrophotometer at 560 nm. Diclofenac (50 mg/mL) was used as reference standard and a control was prepared by omitting the extracts. The experiment was performed in triplicate. The percentage of HRBC membrane stabilization or protection was calculated by using the formula mentioned below:-

$$\text{Percent inhibition} = \frac{\text{Abs. of Control} - \text{Abs. of treated}}{\text{Abs. of Control}} X100$$

In-vitro Anti-arthritic Activity

Inhibition of protein denaturation method

Inhibition of protein denaturation method was performed as described by Kumari *et al*[5]. The suspension test mixture (2.5 ml) consisted of 2.2 ml bovine serum albumin (5% aqueous solution) and 0.3 ml of *S. anquetilia* leaves extract at different concentration levels 100, 200 and 400 mg/mL. The samples were incubated at 37^{0}C for 30 min. After cooling the samples, 7.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured UV spectrophotometer at 660 nm for control test 0.3 ml distilled water was added instead of extracts while product control test lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.

$$\text{Percent inhibition} = \frac{\text{Abs. of Control} - \text{Abs. of treated}}{\text{Abs. of Control}} X100$$

The control represents 100% protein denaturation. The results were compared with acetyl salicylic acid (50 mg/ml) treated samples.

RESULTS

Human red blood cell membrane stabilization method reflects the effect of drugs on cellular membrane i.e. red blood cell. Since HRBC membrane are similar to lysomal membrane components[5,6]. The prevention of hypotoxicity induces HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drugs. The ethyl acetate extract of *S. anquetilia* showed significant anti-inflammatory activity at the concentration of 400 mg/ml which is comparable to the standard drug diclofenac sodium (100 mg/ml). *In-vitro* anti-inflammatory activity of the extracts showed concentration dependent activity.

Table 1: Effect of ethyl acetate extract of *S. anquetilia* leaves (EESA) on HRBC membrane stabilization anti-inflammatory activity

Extracts/ Drug	Concentration (mg/ml)	Percentage inhibitions
Control	----	----
EESA*	50	22.18±0.12
	100	36.11±1.10^b
	200	74.31±2.35
	400	90.70±3.12^a
Diclofenac sodium	100	94.88±2.52^a

*EESA- Ethyl acetate extracts of *S. anquetilia leaves*. Data's statistical significance found as: $^aP< 0.01$, $^bP< 0.05$

Table 2: Effect of ethyl acetate extract of *S. anquetilia* leaves (EESA) on Protein denaturation method for anti-arthritic activity

Extracts/ Drug	Concentration (mg/ml)	Percentage inhibitions
Control	----	----
EESA*	50	28.12±0.18^b
	100	44.35±2.33
	200	72.14 ±3.21^a
	400	92.41±2.4^b
Acetyl salicylic acid	100	96.21±3.4^a

*EESA- Ethyl acetate extracts of *S. anquetilia leaves*. Data's statistical significance found as: $^aP< 0.01$, $^bP< 0.05$

Table 1. Effect of EESA on HRBC Membrane Stabilization

Human red blood cell membrane stabilization results are tabulated in Table 1. The results of 50, 100, 200 and 400 mg/ml of EESA showed concentration dependent activity respectively i.e. 22.18 %, 36.11 %, 74.31 % and 90.70%. Among all the concentration, EESA 100 mg and 400 mg were found statistically significant ($^*P<0.05$). All the results of test drugs were compared with control and standard drug diclofenac sodium which showed 94.88% protection (Table 1).

Table 2. Effect of EESA on Protein Denaturation Method

The production of auto antigens in certain arthritic diseases may be due to denaturation of protein and membrane lysis action. Denaturation of protein causes the production of auto antigens in conditions such as rheumatic arthritis, cancer and diabetes which are conditions of inflammation[7]. Hence, by inhibition of protein denaturation, inflammatory activity can be inhibited. The results of 50, 100, 200 and 400 mg/ml of EESA showed 28.12%, 44.35%, 72.14% and 92.41% activit and among all the concentration, EESA 50 mg and 400 mg were found statistically significant ($^*P<0.05$). The maximum % inhibition of protein denaturation was observed 92.14% at 400 mg/ml and with standard drug acetyl salicylic acid shows 96.21% at 100mg/ml, results are shown in Table 2.

CONCLUSION

The present investigations are scientifically validated *in vitro* using human red blood cell membrane stabilization and protein denaturation assay methods, it supported to traditional claim as

anti- inflammatory and anti-arthritic activity respectively. It can be concluded that the ethyl acetate extract of leaves of *Skimmia anquetilia* can be further used for *in-vivo* activities related to anti- inflammatory and anti-arthritic activity.

CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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