International Journal of Research in Pharmacy and Pharmaceutical Sciences
www.pharmacyjournal.in
ISSN: 2455-698X
Received: 06-02-2024, Accepted: 29-02-2024, Published: 23-03-2024
Volume 9, Issue 1, 2024, Page No. 76-78

In-vitro anti-inflammatory and *anti*-arthritic activities of hydro-alcoholic extract of berries of *Skimmia anquetilia*

Sandeep Goyal[1], Rakesh Chawla[2], Faizul Hasan[3], Vijender Kumar [3*]
[1] Department of Pharmacology, University Institute of Pharmaceutical Sciences & Research, BFUHS, Faridkot, Punjab, India
[2] Department of Pharmaceutical Chemistry, University Institute of Pharmaceutical Sciences & Research, BFUHS, Faridkot, Punjab, India
[3] Department of Pharmacognosy & Phytochemistry, Delhi Pharmaceutical Sciences and Research University, New Delhi, India

Abstract
Skimmia anquetilia is an evergreen, aromatic, monoclinous and perennial shrub. Current investigations *in-vitro* anti-inflammatory and anti-arthritic potentials of berries of *Skimmia anquetilia* were validated with employing the existing well developed models i.e. red blood cells membrane stabilization and protein denaturation methods respectively. Hydro-alcoholic extracts of berries of *S. anquetilia* at different concentration levels (50, 100, 200 and 400 mg/ml) were used and dose dependent inhibition effects on protein denaturation was found 90.11% at 400 mg/ml of extracts and 96.21 % at 100 mg/ml of acetyl salicylic acid as standard in antiarthritic study. Whereas, in membrane stabilization method, maximum effect was found as 89.21 % at 400 mg/ml of extracts and 94.88 % at 100 mg/ml of diclofenac sodium as standard for anti-inflammatory evaluation. Based on the results outcomes, it is concluded that, hydro-alcoholic extract of berries of *S. anquetilia* have shown significant ($^{*a}P<0.05$) potential as anti-inflammatory and *anti*-arthritic effects.

Keywords: *Skimmia anquetilia, in-vitro* anti-inflammatory; *anti*-arthritic activity, membrane stabilization assay, protein denaturation method

Introduction
Inflammation serves as a natural defense mechanism of the body, protecting against infections, irritations, allergens, and other harmful stimuli. It is an integral part of the body's defense mechanisms and plays a crucial role in various inflammatory reactions, including the release of histamine, bradykinin, prostaglandins, fluid leakage, cell migration, tissue breakdown, and repair. These reactions are aimed at defending the body and are typically activated in most disease conditions. Inappropriate inflammation has been increasingly recognized as a critical factor in many diseases that affect humans, such as cardiovascular diseases, inflammatory and autoimmune disorders, neurodegenerative conditions, infections, and cancer [1]. The stabilization of the lysosomal membrane is essential in the inflammatory response. It inhibits the release of lysosomal constituents from activated neutrophils, such as bactericidal enzymes and proteases, which can cause tissue inflammation and damage when released outside the cells. Additionally, stabilizing the lysosomal membrane can have a similar effect on the erythrocyte membrane, as both membranes share analogous properties. Therefore, the stabilization of the human red blood cell membrane through hypotonicity-induced membrane lysis can be used as an *in-vitro* measure to assess the anti-inflammatory activity of unexplored drugs or plant extracts. Skimmia anquetilia (S. anquetilia) is an aromatic shrub that belongs to the Rutaceae family. It is predominantly found in the Western part of the Himalayas and Kashmir in India. Traditionally, the infusion of S. anquetilia leaves has been used for treating headaches, providing a sense of freshness, and alleviating general fever [1, 3]. The leaves of S. anquetilia are known for their aromatic properties and contain compounds such as linalool, geraniol, pinene, scopolamine, skimmianine, and umbelliferone [3]. The present study aims to scientifically validate the *in vitro* anti-inflammatory effects of S. anquetilia through human red blood cell membrane stabilization and assess its anti-arthritic activity using the protein denaturation assay method.

Materials and Methods
Plant material
The berries of *Skimmia anquetilia* were collected from Roadsides part of Gulmarg area of Kashmir (J&K, India). The Selected plant materials were identified by Dr. A. R. Naqshi, Taxonomist, University of Kashmir, Srinagar. The hydroalcoholic extracts were prepared from air dried berries of S. *anquetilia*.

In -vitro anti-inflammatory activity of hydroalcoholic extract of berries of *Skimmia anquetilia* (HEBSA)
HRBC membrane stabilization method
Human red blood cell (HRBC) membrane stabilization method well explored and reliable method for determination of *in -vitro* anti-inflammatory activity as mentioned by Kumar *et al* [4]. The blood was 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 mixture was centrifuged at 3 000 rpm for 20 minutes. The packed RB cells were washed with isosaline and a 10% suspension was made. Hydroalcoholic extracts of berries of S. anquetilia was prepared and evaluated at different concentration levels 100, 200 and 400 mg/mL) using distilled water as solvent and to each test tube 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. It was incubated at 37^0C 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 drug and a control was prepared by omitting the extracts. The experiment was performed in triplicate. The percentage of HRBC membrane stabilization or inflammation protection was calculated by using the formula mentioned below

$$\text{Percent inhibition} = \frac{\text{Abs. of Control} - \text{Abs. of treated}}{\text{Abs. of Control}} \times 100$$

In-vitro anti-arthritic activity of HEBSA

Inhibition of protein denaturation assay method

Inhibition of protein denaturation method was performed as described by Verma etal [5]. The suspension test mixtures (2.5 ml) consisted of 2.2 ml bovine serum albumin (5% aqueous solution) and 0.3 ml of berries of S. anquetilia extract at different concentration levels 100, 200 and 400 mg/Ml were used. The samples were incubated at 37^0C for 30 min. After cooling each samples, 7.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured using 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 described by Kumari *et al* [6].

$$\text{Percent inhibition} = \frac{\text{Abs. of Control} - \text{Abs. of treated}}{\text{Abs. of Control}} \times 100$$

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* source 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 was shown concentration dependent activity.

Effect of HEBSA on HRBC membrane stabilization

Human red blood cell membrane stabilization results are tabulated in Table 1.

Table 1: Effect of hydroalcoholic extract of berries of S. *anquetilia* (HEBSA) on HRBC membrane stabilization anti-inflammatory activity

Extracts/ Drug	Concentration (mg/ml)	Percentage inhibitions
Control	----	----
HEBSA *	50	26.10 ± 0.22
	100	40.17 ± 1.15[b]
	200	79.10 ± 1.15
	400	89.21 ± 2.11[a]
Diclofenac sodium	100	94.88 ± 2.52[a]

*HEBSA- Hydroalcoholic extracts of berries of S. *anquetilia*. Data's statistical significance found as: a P< 0.01, b P< 0.05.

The results of 50, 100, 200 and 400 mg/ml of HEBSA showed 26.10 %, 40.17 %, 79.10 % and 89.21% concentration dependent activity respectively, among all the concentration, HEBSA 100 mg and 400 mg were found statistically significant (^{*a}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).

Effect of HEBSA on Protein denaturation method

The production of auto antigens in certain arthritic disease may be due to denaturation of protein and membrane lysis action.

Table 2: Effect of hydroalcoholic extract of berries of S. *anquetilia* (HEBSA) on Protein denaturation method for anti-arthritic activity

Extracts/ Drug	Concentration (mg/ml)	Percentage inhibitions
Control	----	----
HEBSA *	50	30.22 ± 1.18 [b]
	100	51.25 ± 1.21
	200	76.21 ± 1.11 [a]
	400	90.11 ± 1.33[b]
Acetyl salicylic acid	100	96.21 ± 3.41[a]

*HEBSA- Hydroalcoholic extracts of berries of S. *anquetilia*. Data's statistical significance found as: a P< 0.01, b P< 0.05.

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 HEBSA showed 30.22%, 51.25%, 76.21% and 90.11% concentration dependent activity respectively, among all the concentration, HEBSA 50 mg and 400 mg were found statistically significant (^{*b}P<0.05). The maximum % inhibition of protein denaturation was observed 90.11% 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 hydroalcoholic extract of berries 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.

References

1. Kumar V, Bhat ZA, Kumar D, Khan NA, Chashoo IA. Evaluation of anti-inflammatory potential of leaf extracts of *Skimmia anquetilia*. Asian Pac J Trop Biomed,2012:2(8):627-630.
2. Vadivu R, Lakshmi KS. *In vitro* and *in vivo* anti inflammatory activity of leaves of Symplocos cochinchinensis (Lour) Moore ssp Laurina. Bangladesh J Pharmacol,2008:3:121-124.
3. Kunwar RM, Shrestha KP, Bussmann RW. Traditional herbal medicine in Far-west Nepal: a pharmacological appraisal. J Ethnobiol & Ethnomed,2010:6:35. doi:10.1186/1746-4269-6-35.
4. Kumar V, Bhat ZA, Kumar D, Bohra P, Sheela S. *In-vitro* anti-inflammatory activity of leaf extracts of *Basella alba* linn. var. *alba*. Int. J. Drug. Dev. & Res,2011:3(2):124-127.
5. Verma P, Singh B, Kaur A, Kumar V. *In-vitro* anti-inflammatory and anti-arthritic activities of ethyl acetate extract of *Skimmia anquetilia* leaves. Journal of Medicinal Herbs and Ethnomedicine,2020:6:42-44.
6. Kumari CS, Yasmin N, Hussain MR, Babuselvam M. *In vitro* anti- inflammatory and anti-arthritic property of *Rhizopora mucronata* leaves. International Journal of Pharma Sciences and Research,2015:6:3:482-485.
7. Babuselvam M, Ravikumar S, Mohamed Farook KA, Abideen S, Peer Mohamed M, Uthiraselvam M. Evaluation of anti-inflammatory and analgesic effects on the extracts of different parts of Excoecaria agallocha L. J. Appl Pharma Sci,2012:2(9):108-12.
8. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins, Journal of Pharmacy and Pharmacology,1968:20(3):169-173.