Indian Journal of Traditional Knowledge
Vol 21(3), July 2022, pp 489-498

# Antimicrobial and anti-inflammatory response by two formulations of Jatyadi thailam in healing diabetic foot ulcers

K Swathi[a], S Sumathi[a,]*, Somit Kumar[b] & Shubashini K Sripathi[c]

[a]Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore 641 043, Tamil Nadu, India

[b]AVP Research Foundation, Ramanathapuram, Coimbatore 641 045, Tamil Nadu, India

[c]Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore 641 043, Tamil Nadu, India

E-mail: Sumathi_bc@avinuty.ac.in

*Received 02 June 2021; revised 26 November 2021*

Jatyadi thailam is a polyherbal formulation used by Ayurvedic practitioners and clinically reported for the treatment of inflammation related ailment specifically in non-healing chronic foot ulcers. The study is focused to validate and compare the Ayurvedic Formulary of India (AFI) and Yogagrantha (YG) formulations of Jatyadi thailam *in vitro*, for its antimicrobial and anti-inflammatory potential. Antimicrobial activity of thailam was determined by broth microdilution method for its minimal inhibitory concentration (MIC) and microbicidal activity (MBC/MFC). *In vitro* anti-inflammatory activity of varying concentrations of Jatyadi thailam were determined by assaying albumin denaturation inhibition, membrane stabilization (hypotonicity-induced hemolysis), heat induced hemolysis and antiproteinase activities. Highest bactericidal and fungicidal activity was recorded by AFI formulation of Jatyadi thailam showing low MIC values compared to YG formulation. Of all the tested bacterial strains, both the formulations showed great bactericidal effect against *Staphylococcus aureus*. Both the formulations exhibited significant dose dependent anti-denaturation and anti-proteinase activities. The thailam also showed marked stabilization of human red blood cell (HRBC) membrane against thermal hemolysis. Our study confirmed AFI formulation of Jatyadi thailam possessed better antimicrobial and significant ($p<0.05$) anti-inflammatory effect. AFI Jatyadi thailam was more effective than YG formulation in terms of dose-dependence activity against infection causing microbes and toxic inflammatory mediators. The outcome of the study emphasizes the positive therapeutic potential of Jatyadi thailam to combat infectious and inflammatory conditions.

**Keywords**: Diabetic foot ulcers, Infection, Inflammation, Jatyadi thailam, Polyherbal Ayurvedic formulation

**IPC Code**: Int Cl.[22]: A61K 36/00, A61P 17/00, A61P 17/02

Skin, being the outer covering of the body provides first line of protection against harmful external stimuli and pathogens. Alterations in the local resistance leads to the proliferation of opportunistic pathogens susceptible to topical injuries and several infection associated secondary complications. An infected wound is a localized obstruction of the skin or intrinsic soft tissue wherein the pathogenic species have penetrated into viable tissue surrounding the wound[1]. *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli*, *Proteus* spp., *Klebsiella*, *Enterobacter*, *Pseudomonas aeruginosa*, *Enterococci*, *Clostridium*, *Bacteroides*, *Peptostreptococcus*, *Candida* are the most common pathogens in wound infection. These can seriously affect the normal wound healing process by interrupting normal coagulation processes and facilitating aberrant leukocyte function, which results in the unhealthy granulation tissue formation, decreases the ultimate tensile strength of a wound and adversely affecting epithelialization[2]. Uncontrolled and untreated wound infections in chronic diabetic condition can lead to the more prone risk determinant of gangrene and even amputations of lower extremities[3]. Antibiotics and certain other allopathic drugs can essentially save lives and are helpful in managing diseases caused by infectious microorganisms. Nevertheless, they have the ability to cause deteriorating side effects, like all medications[4]. The prevailing synthetic antibiotic drugs increases multidrug resistance or drug-resistant pathogens to soft tissue infection triggered by bacteria in diabetic patients and causes adverse drug effect to

*Corresponding author

the host immune system[5]. Drug resistant infections are a global problem and the implication that lack of new generation of antimicrobials over the last few decades has sparked an interest towards the use of Complementary and Alternative Medicine (CAM). On the other hand, traditional plant driven formulations and certain natural medicinal products may serve as a safer therapeutic intervention to synthetic drugs to combat infectious diseases, based on its own antimicrobial properties (bactericidal or bacteriostatic)[6].

Infection of the wound activates the body's immune response, causing inflammation which is massively important in eliminating the pathogens; but it may result in tissue impairment, if prolonged. This could result in chronic inflammation, thereby delays the normal healing process[7]. Inflammation is the body's protective mechanism of cascade of events that occurs in response to harmful external stimuli, pathogens and trauma in the biological systems. Inflammation induces a gradual improvement in the normal functioning of the cells at the tissue injury site and is characterized by tissue degradation and recovery from the inflammatory process. Loss of recruitment of monocytic cells for the removal of cell or tissue debris in acute phase simultaneously leads to chronic phase of inflammation[8]. Chronic inflammatory conditions are characterized by redness, pain, heat, swelling and loss of tissue function, arising from local immune, vascular and inflammatory cell responses to contagion or wound. Chronic inflammation causes a serious tissue damage which can also lead to a host of other disease conditions due to interruption of the normal restoration of healthy cells[9]. Inflammation is a non-specific internal defense of our body frequently allied with the surge in vascular permeability, increase of albumin denaturation and membrane destabilization[10]. Certain nonsteroidal anti-inflammatory medications such as Aspirin, Diclofenac, Ibuprofen etc, help relieve discomfort of symptoms and reduce inflammation and pain by obstructing the inflammatory response and enhancing the protein stability[11]. However, risk factors associated to the administration of those drugs becomes a primary concern. Thus, Ayurvedic medicinal system can act as a goldmine for novel anti-inflammatory drugs and offers a natural guard for those with inflammatory illnesses.

Ayurveda is a traditional healing practice endowed with diverse medicinal properties that offers a hallmark of holistic treatment. *Vrana-shotha* (initial stage of inflammation), *Dushta-vrana* (chronic wound), *Svayathu*, *Shothaa*, *Shofa* are the terms used in Ayurveda to refer inflammation[12]. Ayurvedic medicine refers the inflammation as a vascular and cellular response and a complication of degenerative diseases. The body reacts to inflammation by ooze out the cells and tissues and releases protein into the extracellular matrix. This causes congestion in microvascular circularization and affects the diffusion rate of nutrients, oxygen and wastes. The consistent disturbance in the microchannel is responsible for the loss of homeostasis, inflammation and tissue injury that evolves into chronic inflammatory diseases[13]. Medicinal plants possessing antimicrobial, antioxidant and anti-inflammatory properties have lessened the wound healing process. Ayurveda is regarded as "Goddess of All Healing" and is considered to be one of the most powerful traditional medicine systems with ample healing and curing properties. The therapeutic values of medicinal plants depend upon the presence of one or more constituents possessing certain physiological and pharmacological activity[14].

All Ayurvedic formulations encompass active elements of herbal constituents into the liquid sample by various extraction techniques[15]. Jatyadi thailam is a widely used polyherbal ayurvedic drug formulation that is clinically used for topical application for wide variety of skin ailments[16]. The prominence of Jatyadi thailam lies in the fact that it stabilizes all types of doshas -*Vata*, *kapha* and *pitta*. The complex formulation comprises of multiple phytochemical components/minerals that function synergistically or agonistically towards pharmacological action. Since, no scientific data was reported on the underlying mechanism of action of Jatyadi thailam, the present study was intended to evaluate and compare the *in vitro* antimicrobial and anti-inflammatory properties of AFI (Ayurvedic Formulary of India) and YG (Yogagrantha) formulations.

## Materials and Methods

### Solubility of samples

Standardized Jatyadi thailam was prepared as per AFI guidelines by Arya Vaidhya Chikitsalayam, Coimbatore and used for the study. YG formulation of Jatyadi thailam was also prepared and provided by Arya Vaidhya Chikitsalayam by making minor modifications by replacing some of the herbs in the composition without compromising on the efficacy of drug.

Jatyadi thailam was first tested for its solubility using various organic solvent systems. The solvents

tried were hexane, diethyl ether, ethyl acetate, chloroform, acetone, dimethyl sulfoxide (DMSO), ethanol, methanol, water and 50% hydroethanol. A specific volume (1 mL) of the thailam was dispensed in different tubes with equal volume of solvents to be checked for. Also, we attempted to use PEG-40 hydrogenated castor oil (PEG-40 HCO) and SPAN 80 and checked for the solubility. Here, the samples were prepared according to w/v method.

***In vitro* anti-microbial activity**

The antimicrobial activity of Jatyadi thailam was evaluated in terms of bactericidal and fungicidal effect by microbroth dilution method. The organisms chosen were the ones responsible for causing diabetic wounds.

**Microbroth dilution method**

Broth micro dilution method has been used to determine the minimum inhibitory concentrations (MIC) of the samples against gram positive (*Staphylococcus aureus, Bacillus subtilis*) and gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*) diluted in nutrient broth and fungal cultures (*Aspergillus fumigatus, Aspergillus flavus, Candida albicans, Cryptococcus neoformans)* in sabourard dextrose broth.

**Preparation of McFarland standard**

Bacterial and fungal cell suspensions were adjusted to 0.5 McFarland turbidity standards equivalent to $1.5\times10^8$ cells/mL inoculum. The preparation was maintained in an air tight bottle and used for the study.

**Determination of minimum inhibitory concentration (MIC) and minimal bactericidal (MBC)/fungicidal concentration (MFC)**

The antimicrobial activity of the formulations was examined using the broth microdilution method which was used to define the minimum inhibitory concentration (MIC). Broth microdilution method was done according to CLSI standards[17]. Minimal bactericidal concentration (MBC), an antibacterial activity and minimal fungicidal concentration (MFC), an antifungal activity was determined from inoculating the contents from the MIC plates onto the respective medium and the results were observed after 24 h incubation at 37°C. MBC and MFC value was assumed as 99.9% inhibition of bacterial and fungal growth after inoculation in Petri plate. Bacteriostatic and Fungistatic effect was determined as the absence of growing colonies observed after 24 h of incubation, but with visible growth after 48 h incubation.

***In vitro* anti-inflammatory activity**

**Inhibition of albumin denaturation[18]**

The reaction mixture consists of varying concentration of samples (500 µg/mL to 2500 µg/mL) and 1% aqueous solution of bovine albumin fraction; the pH of the reaction mixture was balanced using small amount of 1N HCl. The samples were incubated for 20 min at 37°C and then heated for 30min at 57°C. The turbidity was spectrophotometrically measured at 660 nm after cooling the samples.

**Membrane stabilization test[19]**

RBC Suspension was prepared according to the method of Sadique *et al*.,[20]. The assay mixture consists of 1 mL sodium phosphate buffer (pH 7.4), 2 mL of hyposaline, 0.5 mL human red blood cell (HRBC) suspension, sample of varying concentration ranging from 500 µg/mL to 2500 µg/mL and the final reaction mixture were made upto 4.5 mL with isosaline. Then the mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The released haemoglobin content in the supernatant was read at 560 nm.

**Heat induced hemolysis[21]**

The reaction mixture consists of 1 mL of different concentrations of test sample solution and 1 mL of 10% RBCs; instead of sample only saline was added to the control test tube and incubated at 56°C for 30 min. After cooling, the reaction mixture was centrifuged at 2500 rpm for 10 min and the absorbance of the supernatant was read at 560 nm.

**Proteinase inhibitory action[22]**

The reaction mixture contains 0.06 mg trypsin, 1 mL 20 mM Tris HCl buffer (pH 7.4) and 1 mL varying concentrations of test samples and incubated at 37°C for 5 min. Then added 1 mL of 0.8% (w/v) casein and further incubated for 20 min. To terminate the reaction, 2 mL 70% perchloric acid was added. The cloudy suspension was centrifuged for 10 min at 3000 rpm and the absorbance of the supernatant was read at 210 nm.

**Statistical analysis**

All data of anti-inflammatory activities were expressed as Mean±SD using at least three independent replicates. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Dunnett's multi-comparison test using GraphPad Prism version 8.00 for Windows. A value of $p<0.05$ was considered to be statistically significant.

## Results

### Solubility of Jatyadi Thailam formulations

The present study was formulated to test and compare the effects of AFI and YG formulations of Jatyadi thailam using *in vitro* systems. These systems involve cell-free systems and culture of cells, all of which are aqueous-based test systems. Since both the formulations of Jatyadi thailam is an oil-based formulation, an ideal solubilisation system to meet the aqueous-organic barrier to deliver the components of the thailam to the test system is imperative. Higher the solubility, the larger is the amount of compound that can dissolve in a solution.

From the results obtained using various solvents in the order of increasing polarity, absolute solubility was not attained for both the formulations of Jatyadi thailam. The solvent mixtures showed dispersion of oil components and resulted only in immiscible precipitates. However, the therapeutic effect of the samples can be validated only when it is able to interact with the cell system across the medium. In a nutshell, a solvent that make the samples to become more soluble must be identified. To overcome this problem, we used PEG-40 hydrogenated castor oil (PEG-40 HCO), a non-ionic surfactant in oral, topical and parental drug delivery systems[23]. This was used as a non-ionic oil-in-water solubilizer, to increase the solubility, and to avoid emulsification, SPAN 80 was used to improve the accessibility to transdermal delivery system. Therefore, a combination mixture of PEG-40 HCO, SPAN 80 and water, which resulted in complete solubility was used for further studies.

### Antimicrobial activity by broth microdilution method

The antimicrobial activity of AFI and YG formulations against selected microorganisms was done by microbroth dilution technique. Antimicrobial susceptibility tests were performed to evaluate how effective antimicrobials could be derived from herbal extracts against various pathogenic microorganisms. MIC method was used to assess the efficacy of the formulations to suppress bacterial growth being tested. To investigate whether the formulations are potent enough to inhibit the microorganisms and to prevent infections, MIC was done by using the microdilution broth method in 96 well microplates. The turbidity in the microtitre well could indicate the growth of microorganisms. The lowest concentration of an antimicrobial agent necessary to inhibit the visible growth of bacteria and fungus that showed no turbidity after incubation is considered as MIC. The antimicrobial activity was investigated against infection causing microbes namely *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Aspergillus flavus*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Candida albicans* and the results are depicted in Table 1 and 2. Ciprofloxacin, Streptomycin and Nystatin were used as the reference standards.

Table 1 — MIC and MBC of AFI and YG formulations of Jatyadi thailam against selected gram-negative and gram-positive bacteria by broth dilution method

| Test organisms | AFI Jatyadi thailam | | YG Jatyadi thailam | | Standard antibiotic | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Ciprofloxacin | | Streptomycin | |
| | MIC (mg/mL) | MBC (mg/mL) | MIC (mg/mL) | MBC (mg/mL) | MIC (mg/mL) | MBC (mg/mL) | MIC (mg/mL) | MBC (mg/mL) |
| *Escherichia coli* | 0.625 | 1.250 | 1.250 | 2.500 | 0.062 | 0.062 | 0.062 | 0.062 |
| *Pseudomonas aeruginosa* | 0.625 | 0.625 | 0.625 | 0.625 | 0.125 | 0.125 | 0.062 | 0.062 |
| *Klebsiella pneumoniae* | 0.625 | 1.250 | 1.250 | 2.500 | 0.125 | 0.125 | 0.250 | 0.250 |
| *Bacillus subtilis* | 1.250 | 2.500 | 1.250 | 2.500 | 0.062 | 0.062 | 0.125 | 0.125 |
| *Staphylococcus aureus* | 0.312 | 0.312 | 0.625 | 1.250 | 0.031 | 0.031 | 0.062 | 0.062 |

Table 2 — MIC and MFC of AFI and YG formulations of Jatyadi thailam against selected fungal organisms

| Test organisms | AFI Jatyadi thailam | | YG Jatyadi thailam | | Standard antibiotic | |
|---|---|---|---|---|---|---|
| | | | | | Nystatin | |
| | MIC (mg/mL) | MFC (mg/mL) | MIC (mg/mL) | MFC (mg/mL) | MIC (mg/mL) | MFC (mg/mL) |
| *Aspergillus fumigatus* | 0.156 | 0.156 | 0.312 | 0.624 | 0.031 | 0.062 |
| *Aspergillus flavus* | 0.312 | 0.312 | 0.625 | 0.625 | 0.125 | 0.250 |
| *Cryptococcus neoformans* | 0.312 | 0.624 | 0.625 | 1.250 | 0.125 | 0.250 |
| *Candida albicans* | 0.625 | 1.250 | 1.250 | 2.500 | 0.500 | 0.500 |

**Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal (MBC)/Minimal Fungicidal (MFC) Concentration**

The results of the minimum inhibitory concentration (MIC) using broth microdilution method (Table 2) revealed that both gram-positive and gram-negative bacteria tested were susceptible to both the formulations. The YG Jatyadi thailam had the lowest activity compared to the AFI Jatyadi thailam but also exhibited better antibacterial effect. The MIC values in the broth dilution by AFI formulation was found to be 0.625 mg/mL for all the gram-negative bacteria and 1.250 mg/mL for *B. subtilis* and 0.312 mg/mL for *S. aureus* with MBC values ranged from 0.312 mg/mL to 2.500 mg/mL. From the result, it was observed that among all the organisms tested, both the formulations exhibited its best antibacterial activity against the gram-positive *S. aureus.* The standard drugs ciprofloxacin and streptomycin showed good antibacterial activity with MIC value ranging from 0.031 mg/mL to 0.125 mg/mL in the broth dilution. Ciprofloxacin and Streptomycin inhibited all the organisms tested.

The results of antifungal activity of both the formulations as shown in Table 2 confirmed that the AFI Jatyadi thailam had MIC of 0.312 mg/mL (*A. flavus, C. neoformans*) and 0.625 mg/mL (*C. albicans)* while it showed a MIC value of 0.156 mg/mL on *A. fumigatus* exhibiting its best activity. The results of YG formulation of Jatyadi thailam were similar to that of first observation except the variations in MIC values which ranged from 0.312 mg/mL to 1.25 mg/mL. However, a very high susceptibility was observed by AFI Jatyadi thailamin all the tested fungal isolates, with much better activity for *A. fumigatus* with a MFC value of 0.156 mg/mL. Nystatin also showed a high antifungal activity on the tested organisms with MIC ranging from 0.03 mg/mL to 0.500 mg/mL. Therefore, on comparing the results, AFI Jatyadi thailam was more active than YG formulation in affording the bactericidal and fungicidal effect.

**Anti-inflammatory activity**

**Jatyadi thailam inhibits albumin denaturation**

Albumin denaturation is a known cause of inflammation. Protein denaturation is a primary cause of inflammation that leads to lose of stability of protein structure by external stress and chemical agents that cause modifications in electrostatic interactions, hydrophobic and disulphide bonding[24]. Inhibition of protein denaturation has been used as a convenient tool to check the protection rendered by Jatyadi thailam to the denaturation process and the results are depicted in Figure 1. The experimental data showed that both AFI and YG formulations of Jatyadi thailam inhibited albumin denaturation in a concentration-dependent manner. Maximum inhibition of 91.05±0.70% was observed from AFI Jatyadi thailam followed by YG Jatyadi thailam (52.24±0.01%) at a highest concentration of 2500 µg/mL. Both the formulations of thailam revealed statistically significant inhibitory ($p<0.05$) activity at all the concentrations tested compared to the control. The results were compared with the standard anti-inflammatory drug aspirin which showed 95.53±0.70% inhibition of protein denaturation at its lowest concentration of 500 µg/mL. The AFI formulation of thailam was found to be more potent ($p<0.05$) in inhibiting the denaturation of albumin when compared to the YG formulation, thereby rendering efficient protection to the biological function.

**Jatyadi thailam exhibits membrane stabilization effect**

Stabilization of the human red blood cell membrane is an important criterion in restraining the inflammatory reaction by inhibiting the release of activated neutrophil lysosomal constituents, including bacterial enzymes and proteases, which trigger tissue damage to extracellular release[25]. When RBC is exposed to hypotonic stress, the release of haemoglobin from RBC is deterred by anti-inflammatory agents because of membrane stabilization. Since, the erythrocyte membrane is homologous to the lysosomal membrane components[26], HRBC membrane stabilization by drugs against hypotonicity-induced hemolysis acts as a key in the analysis of anti-inflammatory activity.

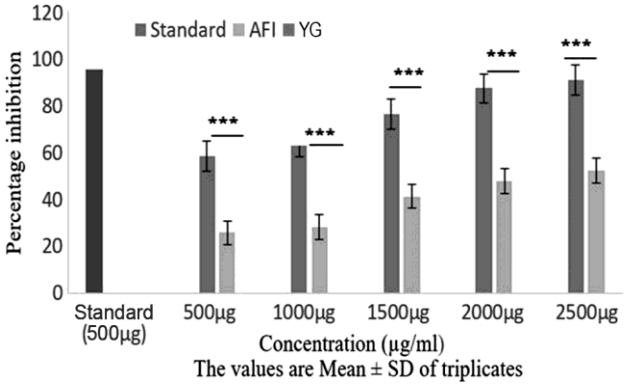


Fig. 1 — Inhibition of albumin denaturation (***$p<0.001$; N=3)

From the results (Fig. 2), it can be interpreted that AFI Jatyadi thailam showed a dose dependent increase in percentage inhibition ranging from 47.83±0.23% to 68.85±0.17% compared to YG formulation which ranged between 32.66±0.55% to 52.29±0.68% for the concentration ranging from 500 µg/mL to 2500 µg/mL. It was compared with the standard aspirin which showed 90.45±0.48% protection at 500 µg/mL. Thus, both AFI and YG formulations rendered noteworthy protection to the human red blood cell membrane and are statistically significant ($p<0.05$) against the control. However, AFI Jatyadi thailam exhibited a significant ($p<0.05$) membrane stabilization effect compared to the YG Jatyadi thailam. Therefore, both the formulations may render significant protection and stabilization of HRBC membrane. However, AFI formulation may inhibit the processes with much higher efficiency than YG, which may induce or suppress the intracellular efflux[27].

**Jatyadi thailam inhibits heat induced hemolysis**

Red blood cell when exposed to harmful substances like heat causes membrane lysis followed by oxidation and hemoglobin lysis[28]. The capability of the sample to inhibit the membrane lysis induced by thermal treatment was evaluated using HRBC membrane. Both AFI and YG Jatyadi thailam significantly inhibited the hemolysis at all the concentrations tested and the percentage inhibition is shown in Figure 3. The results were comparable to that of standard drug aspirin (89.99±0.02% at 500 µg/mL concentration). From the results, it is clear that, AFI Jatyadi thailam significantly inhibited heat-induced hemolysis ($p<0.05$) more effectively in a dose-dependent manner when compared to the YG Jatyadi thailam. Thus, the inhibition of heat induced HRBC membrane lysis by both the formulation of Jatyadi thailam increased with increasing concentration. This effect may impede the release of lysosomal content of neutrophils, at the inflammatory site, which in turn reduces the tissue damage and hence, the inflammatory response[29]. These results may be a supportive-evidence for membrane stabilization, as well as an added mechanism for the anti-inflammatory properties of Jatyadi thailam.

**Proteinase inhibitory activity**

During inflammation, lysosomal granules of neutrophils carry proteinases[20]. Proteinases has a vital role in the progression of tissue damage and proteinase inhibitors should provide a substantial degree of defense[30]. On increasing the concentration of the thailam from 500 µg/mL to 2500 µg/mL, there was a subsequent increase in proteinase inhibition by AFI Jatyadi thailam where the percentage inhibition ranged between 32.33±0.002% to 74.35±0.005% and in case of YG Jatyadi thailam, it ranged between 25.75±0.39% to 60.50±0.50% (Fig. 4). The results were comparable to the standard drug aspirin that caused 90.96±0.05% inhibition at a concentration of

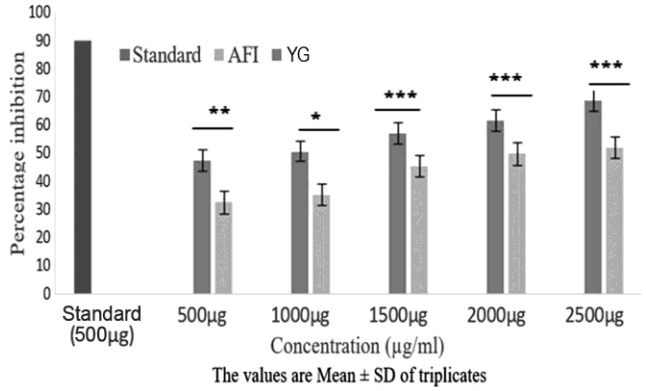


Fig. 2 — Membrane Stabilization Test (***p≤0.001, **p≤0.01, *p≤0.05; N=3)

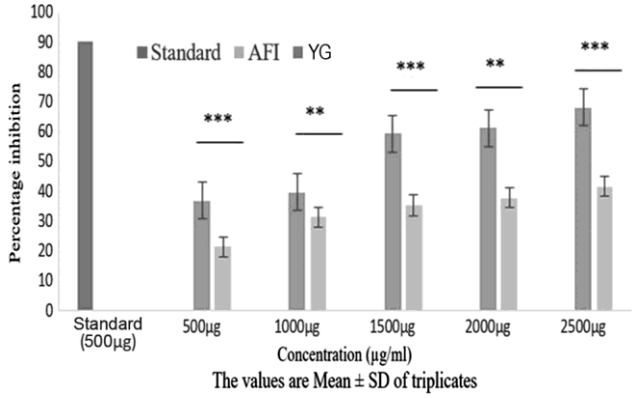


Fig. 3 — Heat Induced Hemolysis (***p≤0.001, **p≤0.01; N=3)

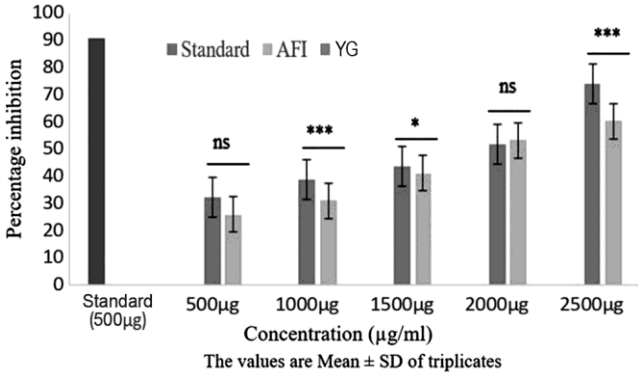


Fig. 4 — Proteinase inhibitory activity (***p≤0.001, *p≤0.05, $^{ns}$p>0.05; N=3)

500 µg/mL. From the result, it was observed that there was no significant difference between these two formulations at concentrations 500 µg/mL and 2500 µg/mL; however, AFI Jatyadi thailam showed superior anti-proteinase activity in terms of concentration dependent inhibition compared to YG Jatyadi thailam.

**Discussion**

Foot ulcers are one of the serious threats of diabetes. Infection is the major cause for lower limb amputation in diabetics. Microbes entering the open wound develops a microbial layer, which makes the infections very difficult to resolve, impairing the host immune system and healing process[31]. Diabetic foot infections are predominantly polymicrobial and the most frequent microorganism isolated so far is *Staphylococcus aureus.* Our results showed both the AFI and YG formulations exhibited great bacteriostatic and bactericidal effect against all the tested microbes especially against gram-positive *Staphylococcus aureus*. This is due to the presence of outer peptidoglycan layer in gram-positive bacteria, that makes the cell wall further responsive to antimicrobials than lipopolysaccharide coat. *S. aureus* is reported to be the single most common isolate (76%) in diabetic wounds and foot ulcers, contributing to variations in wound healing[32] and also the virulence and toxicogenic effects of *S. aureus* in diabetic foot infections are well implicated in most of the studies. Thus, the selected Ayurvedic formulations of Jatyadi thailam showed great antibacterial activity against the common gram-positive *S. aureus* and the wound infection causing pathogens and the efficacy of AFI formulation was better compared to YG formulation.

The AFI Jatyadi thailam exhibited effective fungicidal activity than YG Jatyadi thailam on all tested fungal species. This implies that AFI formulation has more potent bioactive compounds and minerals that may enhance the antimicrobial effect through decreasing the MIC value. This assertion may probably be applied to a point stating plants with more medicinally active compounds may possess low MIC value when compared with plants possessing fewer active compounds[33]. Our results are in accordance with the study of Carson *et al.*[34] showed a potent broad spectrum bactericidal and fungicidal activity of *Melaleuca alternifolia* (Tea Tree) oil. A recent study by Orchard *et al.*[35] on 59 commercial essential oils revealed promising antimicrobial activity against pathogens involved in dermatological infections. The higher effectivity of AFI formulation in our study may be attributable to the modifications in the composition of herbs, which may change the bioefficacy of the polyherbal components towards its pharmacological action. Thus, Jatyadi thailam offers a promising antimicrobial formulation against skin and wound infections.

Inflammation is a natural protective response mediated by complex factors, if unresolved will result in chronic inflammatory conditions and other complications. Certain inflammatory conditions cause auto antigen production which may be due to denaturation of proteins *in vivo*. Protein denaturation may trigger delayed type hypersensitivity as like the native proteins and some anti-inflammatory drugs like flufenamic acid and phenylbutazone, have also been known to cause thermal induced protein denaturation and thus increase inflammation[36]. AFI Jatyadi thailam mediated the inhibition of albumin denaturation more effectively than YG formulation in a concentration-dependent way. Our results are in line with the findings, where an Ayurvedic formulations exhibited protectivity against albumin denaturation[37].

Cells can accomplish their effective function, only if the cellular membrane is intact. Membrane proteins regulates the volume and water content of cells by managing the exchange of sodium and potassium ions. This function will be affected by membrane injury[38]. When red blood cells (RBCs) are exposed to harmful substances like hypotonic medium, heat and methyl salicylate, it results in membrane lysis along with haemolysis and haemoglobin oxidation[28]. During increased permeability prompted by inflammatory mediators, membrane stabilization helps prevent serum protein and fluid leakage into the tissues[39]. With this consideration relating to our study, it can be inferred that Jatyadi thailam possibly inhibits the release of neutrophils' lysosomal content significantly ($p<0.05$) and at the site of inflammation in terms of increase in dose-dependent inhibition. This may be due to the presence of active constituents in these traditional polyherbal formulations against inflammation[40], thereby offering protection to the membrane. AFI Jatyadi thailam was observed to possess significant ($p<0.05$) membrane protection activity when compared to YG Jatyadi thailam. Our results are supported by a study of Mahadevan *et al.*[41] who reported the potential of Dasamula (ayurvedic

formulation) to prevent the lysis and improves the stabilisation of the membrane, thereby exhibiting the anti-inflammatory activity.

Proteinases are enzymes that catalyse the hydrolysis of the protein peptide bonds and thus bring about structural and functional changes in them. Proteinase leukocytes have been suggested to play a significant role in tissue injury during inflammatory processes and proteinase inhibitors must provide a significant level of defense[42]. Therefore, the anti-inflammatory drugs must offer anti-proteinase activity. It can be inferred from our study that AFI formulation has shown significantly higher proteinase inhibition compared to YG Jatyadi thailam and no significant difference was found between the two formulations at 500 µg/mL and 2000 µg/mL concentrations. Diverse studies have shown that flavonoid contents and bioactive compounds present in these kinds of formulations may be contributable to their anti-inflammatory activity. Our findings correlated with a study reported by Dadoriya *et al.*[43] on the anti-proteinase activity of an ayurvedic formulation *Trayodashang Guggulu*.

However, in all these studies, AFI formulation of Jatyadi thailam was found to be more effective than YG formulation in rendering the biological protection to the membrane against thermal denaturation of proteins and proteinase enzyme. The better activity of AFI formulation may be due to the presence of more bioactive components in the thailam that may increase the therapeutic efficacy towards chronic inflammation.

## Conclusion

Both the formulations of Jatyadi thailam (AFI and YG) exhibited the antimicrobial and anti-inflammatory properties. However, on comparing both, AFI Jatyadi thailam exhibited higher susceptibility and effectiveness against infection causing microbes and toxic inflammatory mediators. The difference in the bioefficacy may be explicable owing to the variation in the composition of exotic medicinal plants/bioactive components in the formulation which may possess diverse mechanism of action to exert its anti-infective and anti-inflammatory activity. Moreover, these studies shed a light for further research to see the toxicological properties and underlying signalling pathways in chronic wound-healing. These observations have significant implications, as inflammation at the wound site of non-healing diabetic wounds can interfere and confound with the wound-healing treatment measures undertaken. These positive findings provide scientific evidence that may validate the traditional use of Jatyadi thailam in the treatment of infection and inflammatory diseases.

## Acknowledgement

We deeply acknowledge late Dr. P. R. Padma for her initiation of the project work, constant guidance and encouragement to carry out the research. We also thank Arya Vaidhya Chikitsalayam for providing the standardized formulation of Jatyadi thailam.

## Financial support

The authors are thankful to The Ministry of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) for their funding to carry out the Extra Mural Research Project No. Z.28015/02/2017-HPC (EMR)-AYUSH-A.

## Conflict of Interest

Authors declare that there is no conflict of interest.

## Authors' Contributions

All authors contributed equally to the work.

## References

1 Mummed B, Abraha A, Feyera T, Nigusse A & Assefa S, *In vitro* antibacterial activity of selected medicinal plants in the traditional treatment of skin and wound infections in eastern Ethiopia, *Bio Med Res*, (2018) 1862401 (8).

2 Annan K & Houghton P J, Antibacterial, antioxidant and fibroblast growth stimulation of aqueous extracts of *Ficus asperifolia Miq*. and *Gossypium arboreum* L., wound-healing plants of Ghana, *J Ethnopharmacol*, 119 (1) (2008) 141-144.

3 Powers C A, Diabetes mellitus infections: Harrison's principles of internal medicine, (McGraw Hill publishers Pvt Ltd.), 16th ed., 2004, 2168-2169.

4 Richard A, Gleckman & John S, Antibiotic side effects, *Semin Respir Crit Care Med*, 21 (1) (2000) 0061-0070.

5 Shokeen P, Bala M & Tondon V, Evaluation of the activity of 16 medicinal plants against *Neisseria gonorrhoeae*, *Int J Antimicrob Agents*, 33 (1) (2009) 86-91.

6 Heusser P, Physiologische grundlagen der gesundheitsförderung und das anthroposophisch-medizinische Konzept. In: *Gesundheitsförderung eine neue Zeitforderung Interdisziplinäre Forschung und Beitrag der Komplementärmedizin*, edited by Heusser P, Vol. 6. (Bern, Switzerland: Peter Lang), 2002, pp. 101-129.

7 Zhao R, Liang H, Clarke E, Jackson C & Xue M, Inflammation in Chronic Wounds, *Int J Mol Sci*, 17 (12) (2016) 2085.

8 Ahmed A, An overview of inflammation: Mechanism and consequences, *Front Biol*, 6 (4) (2011) 274-281.

9 Shrivastava A K, Singh H V, Raizada A, Singh S K, Pandey A, *et al.*, Inflammatory markers in patients with rheumatoid arthritis, *Allergol Immunopathol (Madr)*, 43 (1) (2015) 81-7.

10 Gerard J T & Sandra R, Principles of Anatomy and Physiology, (Harper Collins College Publishers), 7th ed, 1993, pp 695.

11 Muzammil S, Manikandan M, Jafar A, Sakthivel P, Geetha S *et al*., Anti-inflammatory studies on *Acalypha indica L*. leaves by membrane stabilization, *Indian J Nat Prod Resour*, 5 (2014) 195-197.

12 Dutta Shastri K A, Hindi commentary on *Sushruta Samhita*, (Published by chaukhamba sanskri sansthan Varanasi), 13th ed. 2002, p. 73.

13 Vinaya B, Inflammation in Ayurveda and modern medicine, *Int Ayu Med J*, 4 (1), (2013) 1-7.

14 Evans W C, Trease & Evans' Pharmacognosy, (16th ed. London: WB Saunders Company Ltd); 2008

15 Prerna & Vidhu, A, Physicochemical and marker based optimization of fermentation process of *Drakshasava*, an Ayurvedic polyherbal formulation, *Indian J Tradit Know*, 19 (1) (2020) 120-127.

16 The Ayurvedic Formulary of India, Part-I, Government of India, Ministry of Health and Family Welfare, Department of Indian System of Medicines and Homoeopathy, 2nd Ed., Delhi, Controller of Publications, Civil Lines, (2003) pp. 388-389.

17 CLSI, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard, 9th ed., CLSI document M07-A9, Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, 2012

18 Sakat S, Juvekar A R & Gambhire M N, *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn., *Int J Pharm Pharmacol Sci*, 2 (2010) 146-155.

19 Sadique J, Al-Rquobahs W A & Bughaith Ei Gindi A R, The bioactivity of certain medicinal plants on the stabilization of RBS membrane system, *Fitoterapia*, 60 (1989), 525-532.

20 Gupta V, Chauhan S, Prakash A & Mathur A, Evaluation of *in vitro* and *in vivo* anti-inflammatory activities of Parthenium camphora, *Recent Res Sci Technol*, 5 (2013) 33-39.

21 Shinde U A, Kulkarni K R, Phadke A S, Nair A M, Dikshit V J, *et al*., Mast cell stabilizing and lipoxygenase inhibitory activity of *Cedrusdeodaraz* (Roxb.) Loud. Wood Oil, *Indian J Exp Biol*, 37 (1999) 258-261.

22 Oyedepo O O & Femurewa A J, Anti-protease and membrane stabilizing activities of extracts of *Fagrazanthoxiloides*, *Olaxsub scorpioides* and *Tetrapleura tetraptera*, *Int J Pharmacogn*, 33 (1995), 65-69.

23 Waghmare N, Waghmare P, Wani S & Yerawar A, Development of isotretinoin gel for the treatment of acne vulgaris, *Res J Pharm Biol Chem Sci*, 2 (1) (2011) 220-230.

24 Sen S, Chakraborty R, Maramsa N, Basak M & Deka S, *In vitro* anti-inflammatory activity of *Amaranthus caudatus* L leaves, *Indian J Nat Prod Resour*, 6 (2015) 326-9.

25 Murugasan N, Vember S & Damodharan C, Studies on erythrocyte membrane: *In vitro* haemolytic activity of Oleander extract, *Toxicol Lett*, 8 (1981) 33-38.

26 Chou C T, The anti-inflammatory effect of *Tripterygium wilfordii* Hook on adjuvant-induced paw edema in rats and inflammatory mediators release, *Phytother Res*, 11 (1997), 152-154.

27 Mounnissamy V M, Kavimani S, Balu V & Quine S D, Evaluation of anti-inflammatory and membrane stabilizing properties of ethanol extract of *Cansjera rheedi* J Gmelin, (Opiliaceae), *Iran J Pharmacol Ther*, (2007) 235-237.

28 Iwueke A V, Nwodo O F & Okoli C O, Evaluation of the anti-inflammatory and analgesic activities of *Vitex doinana* leaves, *Afr J Biotech*, 5 (2006) 1929-35.

29 Ferrali M, Signorini C, Ciccoli L & Comporti M, Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenylhydrazine, divicine and isouramil, *Biochem J*, 285 (1992) 295-301.

30 Govindappa M, Naga S S, Poojashri M N, Sadananda T S & Chandrappa C P, Antimicrobial, antioxidant and *in vitro* anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc, *J Pharmacogn Phytother*, 3 (2011) 43-51.

31 Mah T-FC & O" Toole G A, Mechanisms of biofilm resistance to antimicrobial agents, *Trends Microbiol*, 9 (1) (2001) 34-9.

32 Goldstein E J, Citron D M & Nesbit C A, Diabetic foot infections: Bacteriology and activity of 10 oral antimicrobial agents against bacteria isolated from consecutive cases, *Diabetes Care*, 19 (6) (1996) 638-41.

33 Doughari J A, Elmahmood, A M & Manzara S, Studies on the antibacterial activity of root extracts of *Carica papaya* L, *Afr J of Microbiol Res*, 2 (2007), 67-72.

34 Carson C F, Hammer K A & Riley T V, *Melaleuca alternifolia* (tea tree) oil: A review of antimicrobial and other medicinal properties, *Clin Microbiol Rev*, 19 (1) (2006), 50-62.

35 Orchard A, Sandasi M, Kamatou G, Viljoen A, van Vuuren S. The *in vitro* antimicrobial activity and chemometric modelling of 59 commercial essential oils against pathogens of dermatological relevance, *Chem Biodivers*, 14 (2017) e1600218.

36 Jagtap V A, Agasimundim Y S, Jayachandran E & Sathe B S, *In vitro* anti-inflammatory activity of 2-amino-3-(substituted benzylidinecarbo hydrazide)-4,5,6,7 tetrahydrobenzothiophenes, *J Pharm Res*, 4 (2011) 378-379.

37 Sahiti M, Gurupadayya B M & Dinesh T K, Evaluation of *in vitro* anti-inflammatory activity of Trayodashang Guggulu: An ayurvedic formulation in comparison with allopathic drug, *Int J Res Ayurveda Pharm*, 10 (3) (2019) 92-96.

38 Okoli C O, Akah P A, Onuoha N J, Okoye T C, Nwoye A C, *et al*., *Acanthus montanus*: An experimental evaluation of the antimicrobial, anti-inflammatory and immunological properties of a traditional remedy for furuncles, *BMC Complement Altern Med*, 8 (2008) 27.

39 Suchita V G, Mrunal G Sawant, Varsha M J, Vilasrao J K, Nilam S, *et al*., Assessment of *in-vitro* anti-inflammatory activity of *Cynodon Dactylon* and acyclovir showing synergistic effect by albumin denaturation and membrane stabilization assay, *Mod Appro Drug Des*, 1 (2) (2017).

40 Dutt V, Dutt R, Kuma S & Dhar V, Evaluation of analgesic activity of *Solanum platanifolium Sims*, Fruits, *Indian Drugs*, 44 (2007) 405-407.

41 Mahadevan V, Vadivel V & Brindha R, *In vitro* antioxidant and anti-inflammatory activities of aqueous extract of an Ayurvedic formulation Dasamula and its herbal ingredients: A comparative study, *IJGP*, 10 (4) (2016) S211-S218.

42 Joshi B B, Chaudhari M G & Mistry K N, *In vitro* screening of antiinflammatory and anti-diabetic activity of root extract of *Argyreia nervosa*, *J Pharm Biomed Sci*, 37 (37) (2013) 1964-1971.

43 Dadoriya P, Nandan Dey Y, Sharma D, Yadav M, Manish M. Wanjari, Sudesh N. Gaidhani, Subhose, V, *In vitro* anti-inflammatory and antioxidant activities of an Ayurvedic formulation -Trayodashang guggulu, *Journal Herb Med*, 23 (2020) 2210-8033.