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ANTIMICROBIAL AND ANTI-INFLAMMATORY POTENTIAL OF THE LEAF EXTRACTS, COLUMN FRACTIONS AND COMPOUNDS OF THE MEDICINAL PLANT *JASMINUM FLEXILE* VAHL

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ABSTRACT: The medicinal plant *Jasminum flexile* is reported in traditional medicine as an antidiabetic and anti-inflammatory agent. There is no scientific report on the antibacterial and anti-inflammatory activity of extracts of *Jasminum flexile*. This study investigated the antibacterial and anti-inflammatory potential of the hydroethanol extract of leaves of *Jasminum flexile*, its column fractions and constituent compounds. The column fractions MF14, MF9 and MF5 expressed a zone of inhibition of 12 mm against *Streptococcus mutans* and the isolated compound JFLC1 showed a zone of inhibition of 10 mm against *Streptococcus mutans, Escherichia coli,* and *Klebsiella pneumoniae* comparable to that of the standards ampicillin, kanamycin and chloramphenicol respectively at the same concentration. The highest anti-inflammatory potential was found for the column fraction MF3 (55.14%) as assessed from the percentage of haemolysis. The results reveal that the isolated compounds and extracts of leaves of *Jasminum flexile* possess significant antimicrobial and anti-inflammatory potential.

INTRODUCTION: The Jasminum genus is a widespread genera of medicinal plants utilized since ancient times [1]. According to reports, there are 500 species of plants in the Jasminum genus [2]. Among these, roughly 200 species are found in the *Oleaceae* family, including shrubs, vines and trees endemic to tropical and mild temperate climates. A careful evaluation of these species revealed only 89 valid species, of which only 40 species grow in the Indian sub-continent [3]. Different parts of the Jasminum plant (such as leaves, stems, flowers and roots) have been widely used to prepare several folk medicines. The existence of natural compounds in Jasminum species, particularly secondary metabolites, bestow a wide range of medical applications to it [5, 6, 7]. *Jasminum grandiflorum* are one species of this genera that is well investigated for its medicinal potential and constituent metabolites. The various extracts of *Jasminum grandiflorum* leaves showed significant antimicrobial activity.

The chloroform extract of leaves showed a significant zone of inhibition against *Bacillus subtilis* (25mm) and the ethanol extract against *E.coli* (21mm). Diethyl ether extract showed a low zone of clearance (8mm) against *Streptococcus* sp., and ethanol extract showed a low zone of clearance (8mm) against *Psuedomonas aeruginosa* and *Klebsiella pneumoniae* [8].

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The antibacterial activity of the hot ethanol extract of leaves of *Jasminum grandiflorum* (10µg/ml) against *S. mutans* and *L. acidophilus* was shown to be statistically significant (P≤0.05) at lower doses, with MIC values of 6.25 µg/ml and 25 µg/ml, respectively [9]. The fruit methanolic extract showed a significant inhibitory effect against the plant pathogen *Xanthomonas campestris* and the animal pathogen *Aeromonas hydrophila* with a zone of inhibition of 18.33±0.47mm and 13.66±0.47mm at 100µg/ml respectively, which were equivalent to that of the standard [10].

A topical formulation named *Jatyadighrita,* a polyherbal preparation with *Jasminum grandiflorum* is one of the herbs that was analyzed for its anti-inflammatory potential. When compared to the standard diclofenac sodium (33%), the formulation displayed about 50% inhibition of croton oil-induced ear edema [11].

In-vitro and *in-vivo* models were used to study the anti-inflammatory efficacy of the solvent-free methanolic extract of the dried leaves. The anti-inflammatory effects of *Jasminum grandiflorum* leaves are likely related to their high phenolic content (2.25±0.105 mg/l of gallic acid equivalent), reducing power and free radical-scavenging ability [12, 13].

Yet another species of Jasminum, namely *Jasminum flexile* Vahl, cultivated in Asian countries and sub-tropical regions such as France, Italy, Japan, Morocco, and Egypt [4, 14, 15], is widely explored for its medicinal applications and cosmetic preparations [14].

In our previous work on this plant, we reported the physicochemical characteristics, fingerprinting, antibacterial and anti-oxidant studies of the hexane and hydroethanolic extracts of leaves of *Jasminum flexile*. In the present study, we attempt to investigate the antibacterial and anti-inflammatory potential of the hydroethanol extract of leaves of *Jasminum flexile* and its fractionates.

A simple column chromatographic separation method was used to separate compounds from the leaves of the plant. The separated fractions and compounds from the leaves of *Jasminum flexile* were also subjected to antibacterial and anti-inflammatory assays.

MATERIALS AND METHODS:

Collection of Plant Material: The dried plant *Jasminum flexile* was obtained from Arya Vaidhya Pharmacy (AVP Pvt Ltd.), Coimbatore, India.

The samples were authenticated at the Fischer Herbarium (FRC) at the Institute of Forest Genetics and Tree Breeding in Coimbatore, verified by Dr. Kunjikanan, and acknowledged by the Royal Botanical Gardens of the United Kingdom. The specimen was deposited in the FRC and assigned voucher numbers ranging from 2422 to 2424.

Extraction of Plant Material: The pulverized leaves of *Jasminum flexile* were successively extracted with hexane and hydroethanol (90:10 ethanol-water mixture).

Refluxing Method: Crushed leaves (1000 g) of *Jasminum flexile* were extracted sequentially with hexane (1000 ml) and hydro ethanol for 6 hours. The extract was concentrated by the distillation method. The hexane extract concentrate is coded as JAFL (H), while the hydroethanolic extract is designated JAFL (HE). The yield of concentrated extracts was recorded, and the sample was stored for further investigation.

Column Chromatographic Analysis: The concentrate edhydroethanolic extract (37 g) of *Jasminum flexile* JAFL(HE) was made into a slurry and subjected to chromatographic separation over a column of silica gel (100 g) built-in hexane and eluted with (i) hexane, (ii) hexane-ethyl acetate mixtures with increasing amounts of ethyl acetate and (iii) ethyl acetate-ethanol mixtures with an increasing amount of ethanol.

Eluates of 200ml were collected and concentrated. TLC checked the homogeneity of fractions, and similar fractions were mixed. The combined fractions were coded as main fractions MF 1 to MF 25. The chromatographic analysis led to the isolation of five compounds (JFLC1, JFL C2, JFL C3, JFL C4, and JFLC5). Chart 1 represents the outline of the procedure adopted for isolating compounds from hydroethanol extract concentrate of leaves of *Jasminum flexile*.

Table 1 details the solvent system used for the chromatographic separation of the main fractions and isolated compounds.

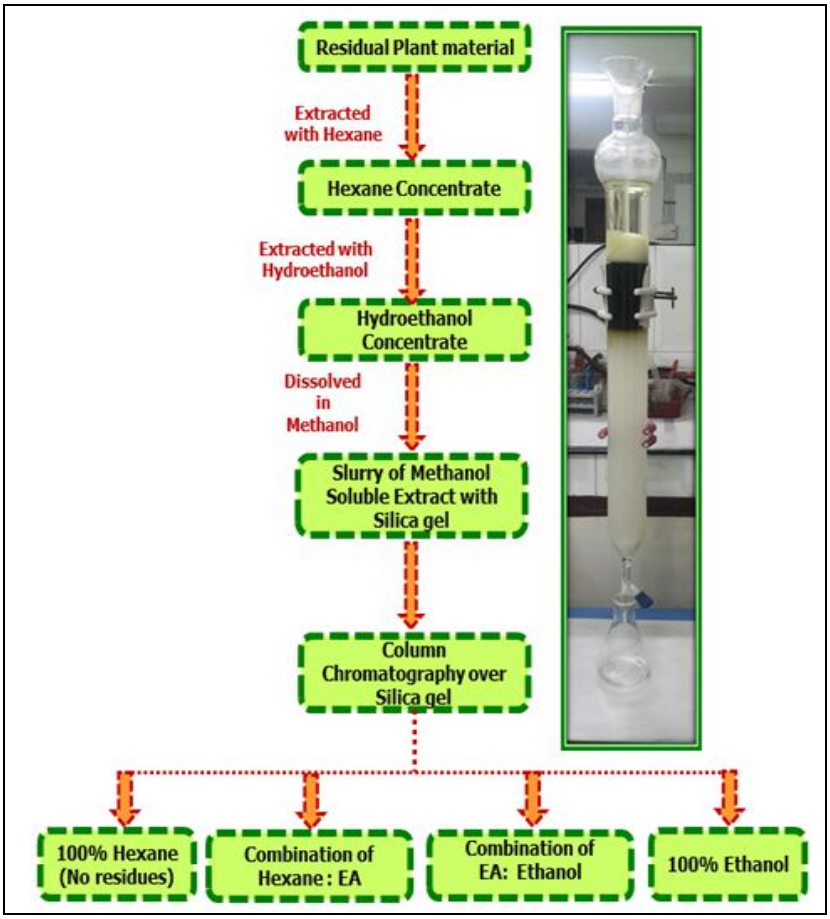

CHART 1: OUTLINE OF PROCEDURE ADOPTED FOR ISOLATING COMPOUNDS FROM HYDROETHANOLIC EXTRACT CONCENTRATE OF LEAVES OF *JASMINUM FLEXILE*

TABLE 1: DETAILS OF ELUENT USED AND FRACTIONS OBTAINED

S. no.	Eluents (v/v)	Column Main Fractions	Isolated Compounds
1	80:20 (H: EA)	MF 1	JFLC1
2	75:25 (H: EA)	MF 2	--
3	70:30 (H: EA)	MF 3	--
4	50:50 (H:EA)	MF 4	--
5	100% EA	MF 5	--
6	95:5 (EA:EtOH)	MF 6	--
7	90:10 (EA:EtOH)	MF 7	JFLC2
8	90:10 (EA:EtOH)	MF 8	--
9	85:15 (EA:EtOH)	MF 9	--
10	85:15 (EA:EtOH)	MF 10	--
11	85:15 (EA:EtOH)	MF 11	--
12	80:20 (EA:EtOH)	MF 12	JFLC3
13	80:20 (EA:EtOH)	MF 13	--
14	80:20 (EA: EtOH)	MF 14	JFLC4
15	70:30 (EA: EtOH)	MF 15	JFLC5
16	70:30 (EA:EtOH)	MF 16	--
17	70:30 (EA:EtOH)	MF 17	--
18	60:40 (EA:EtOH)	MF 18	--
19	50:50 (EA:EtOH)	MF 19	--
20	40:60 (EA:EtOH)	MF 20	--
21	30:70 (EA:EtOH)	MF 21	--
22	20:80 (EA:EtOH)	MF 22	--
23	10:90 (EA:EtOH)	MF 23	--
24	100% Ethanol	MF 24	--
25	100% Ethanol	MF 25	--

*H – Hexane; EA – Ethyl Acetate; EtOH – Ethanol; MF – Main Fraction.

Antibacterial Activity [16]: The antibacterial activity of the fractions and isolated compounds were individually tested against gram-negative and gram-positive microorganisms by agar well diffusion assay. The samples were dissolved in DMSO (dimethyl sulfoxide), sterilized, filtered using a sintered glass filter, and stored at 4°C. The microorganisms were grown overnight in Mueller-Hinton Broth at 30 °C.

The 100 µl of bacterial suspension was spread on the surface of Mueller-Hinton agar plates containing 10^6 CFU ml^{-1} of bacteria. Twenty-five microlitres of each sample (2 mg/ml) were dropped in a 6mm well. Antibiotics ampicillin, kanamycin, and chloramphenicol (2 mg/ml) were positive controls. DMSO was used as a negative control. The plates were incubated for 24 h at 37 °C, and the zone of inhibition was measured. The experiment was done in triplicate.

Anti-Inflammatory Assay- Haemolysis Method [17]: Fresh blood was collected from healthy volunteers and mixed with an equal volume of sterilized Alsever's solution, which serves as an anticoagulant (containing dextrose (2%), sodium citrate (0.8%), citric acid (0.9%) and sodium chloride (0.72%).

The supernatants were removed carefully after centrifuging the blood samples at 10,000 rpm for 15 min at room temperature. The red blood cells were washed in fresh normal saline (0.85% NaCl). The centrifugation and washing process was repeated until the supernatants were clear. The collected RBC suspensions were used for the anti-inflammatory assay. Various concentrations of sample and control were separately mixed with 2ml of phosphate buffer, 4 ml of hypo saline and 0.9ml of RBC suspension. All the assay mixtures were incubated at 37 °C for 30 min. The supernatant liquid was decanted. The haemoglobin content was spectro-photometrically estimated at 620nm. The percentage haemolysis, which is an indication of the anti-inflammatory potential of the plant extract, was calculated using the formula;

$$\% \text{ Haemolysis} = T/C \times 100 \quad (1)$$

T-hemoglobin content of test sample, C-Haemoglobin content of the control.

RESULTS AND DISCUSSION:

Antibacterial Activity of *Jasminum flexile*: The results of the antibacterial activity of the column main fractions and compounds of *Jasminum flexile* as evaluated by agar well diffusion assay are presented in Table 3. In all the samples tested, a zone of inhibition of 10-14 mm was recorded for positive control, whereas no zone of inhibition was observed for DMSO-treated wells. There are differential activities observed for all the tested fractions. Among the 25 fractions (MF1 – MF25) tested, a maximum zone of inhibition of 12 mm for fractions MF5, MF9, and MF14 and a zone of inhibition of 11mm for fraction MF15 and total extract JAFL (HE) were recorded against *S. mutans* in comparison with positive control chloramphenicol which gave 13mm zone of inhibition at the same concentration. Fractions MF6, MF7, MF10, MF14, and JAFL (HE) extract were also active against *E. coli.* Most of the fractions are moderately active against *B. subtilis.* **Fig. 1** represents the photographs of the zone of inhibition for column fractions and compounds of *Jasminum flexile.*

TABLE 2: ANTIBACTERIAL SCREENING RESULTS FOR COLUMN FRACTIONS AND ISOLATED COMPOUNDS OF *JASMINUM FLEXILE* LEAVES

Column Fractions and Compounds Isolated	Zone of Inhibition against test organisms (mm)			
	E. coli	***K. pneumoniae***	***S. mutans***	***B. subtilis***
JAFL (HE)	6	7	8	8
MF 1	8	10	7	5
JFL C1	10	10	10	0
MF 2	0	8	5	2
MF 3	4	10	6	8
MF 4	3	10	10	9
MF 5	8	10	12	4
MF 6	11	8	8	9
MF 7	10	0	8	3
JFL C2	10	10	3	4
MF 8	8	6	6	9

MF 9	9	7	12	0
MF 10	10	4	9	3
MF 11	0	10	8	0
MF 12	0	8	8	4
JFL C3	4	10	9	2
MF 13	8	9	9	2
MF 14	10	8	12	0
JFL C4	12	5	10	4
MF 15	0	6	11	3
JFL C5	8	6	8	6
MF 16	8	7	8	4
MF 17	3.4	2.4	2.8	2.6
MF 18	2	2	2	2
MF 19	2	2	2	1.6
MF 20	1.2	1.6	1	1.2
MF 21	2.2	2	2	3.4
MF 22	2	2	1.6	2
MF 23	1.4	0	2	1.6
MF 24	1.2	1	0.8	1.2
MF 25	1.2	0.4	1	1
Standard Drug used	Ampicillin	Kanamycin	Chloramphenicol	Chloramphenicol
	14	12	13	10
Negative control	0	0	0	0

Sample concentration: 2 mg/ml; Standard concentration:2 mg/ml.

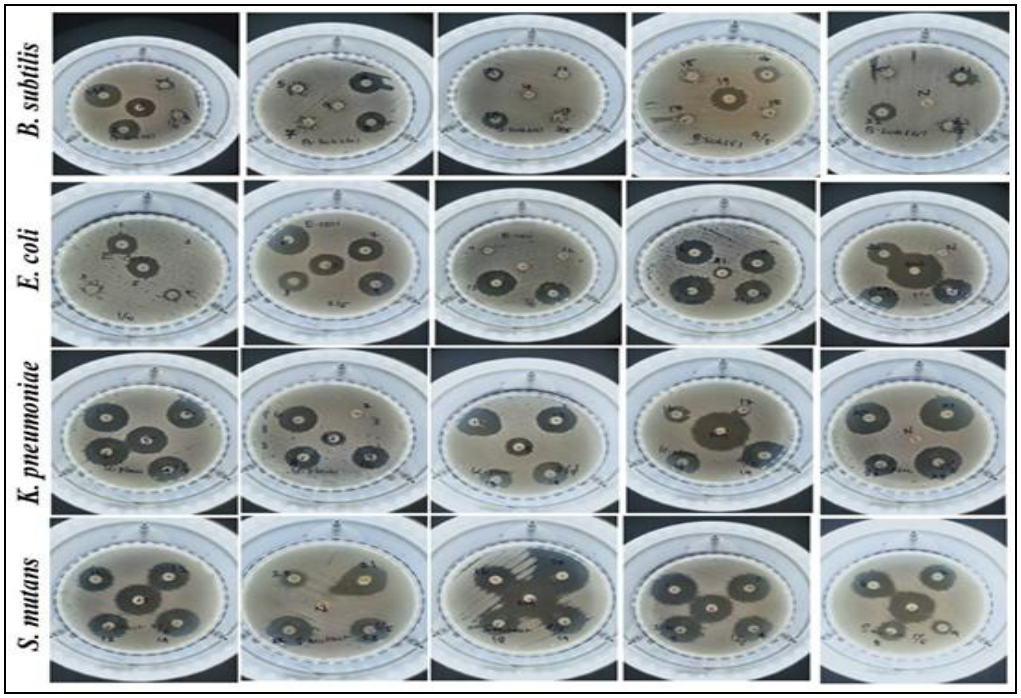

FIG. 1: PHOTOGRAPHS OF THE ZONE OF INHIBITION FOR COLUMN FRACTIONS AND COMPOUNDS OF *JASMINUM FLEXILE*

Anti-inflammatory Activity of *Jasminum flexile:* The anti-inflammatory activity of all the fractions and compounds was evaluated by haemolysis assay. In the hydroethanolic extract, various concentrations of each fractionates and isolated compounds were exposed to pre-prepared red blood cells and the results were expressed in terms of percentage inhibition. The percentage inhibition is indicated by the percentage haemolysis expressed by the samples, which indicates their anti-inflammatory potential. Generally, all the samples tested exhibited an anti-inflammatory effect in a dose-dependent manner. The dewaxed hydroethanolic extract expressed an anti-inflammatory potential of 51.4% at a concentration of 1000 ppm. At the same concentration, the

column main fractions MF 2, MF 5, MF 6, MF 9, MF 14, MF 15, MF 19, and MF 24 showed a higher percentage of anti-inflammatory activity than the parent extract. This indicates that these fractions possess bioactives that exhibit higher synergistic anti-inflammatory activity. The column main fractions MF 4, MF 8, MF 10, MF 13, MF 16, MF 18, MF 20, MF 23, and MF 25 expressed anti-inflammatory activity comparable to the parent extract. At the same concentration, the compounds isolated also showed considerable anti-inflammatory activity. The column fraction MF 6 exhibited good inhibition even at a low concentration of 200 ppm (16.4 % - 200µg/ml), followed by MF 5 (14.26 % - 200µg/ml). Among the isolated compounds, the compound JFL C1 expresses 48.54 % anti-inflammatory activity, followed by JFL C5 (46.82) and JFL C2 (46.16). The results of the *in vitro* anti-inflammatory potential of the various fractions of the leaves of *Jasminum flexile* indicate that most of the fractions contain potentially active anti-inflammatory compounds. The compound JFL C1 tested positive for steroids, whereas JFL C2 tested positive for terpenoids. Compound JFL C3, JFL C4, and JFL C5 have been sugar alcohol moieties. The complete characterization of the compounds will be presented in our next paper. Charts 2 and 3 represent the comparison of the anti-inflammatory activity of the parent extract; the fractionates and compounds isolated from the leaves of *Jasminum flexile*at five different concentrations.

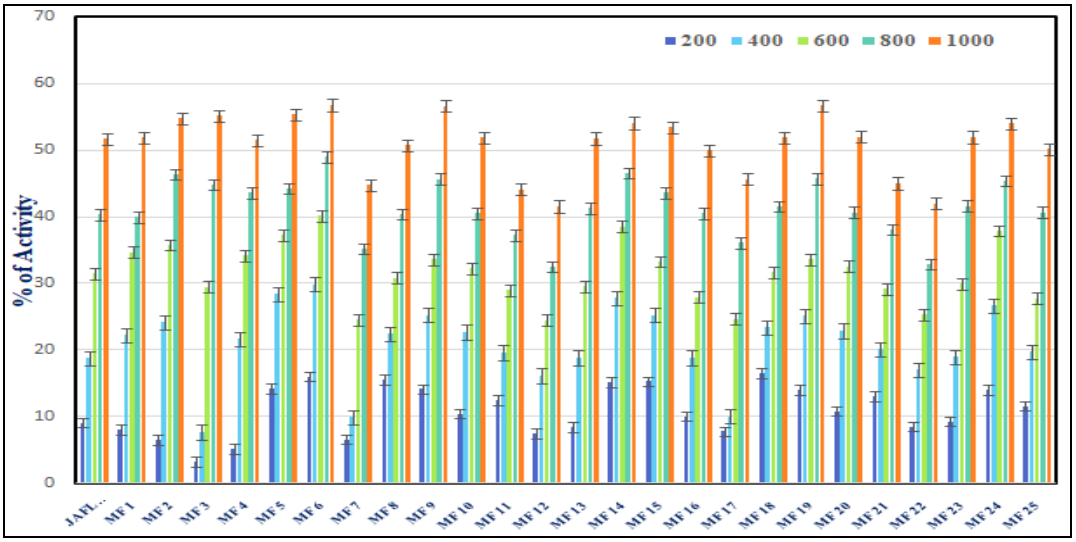

CHART 2: COMPARISON OF ANTI-INFLAMMATORY ACTIVITY OF THE FRACTIONS OBTAINED BY COLUMN CHROMATOGRAPHIC ANALYSIS OF THE LEAVES OF *JASMINUM FLEXILE*

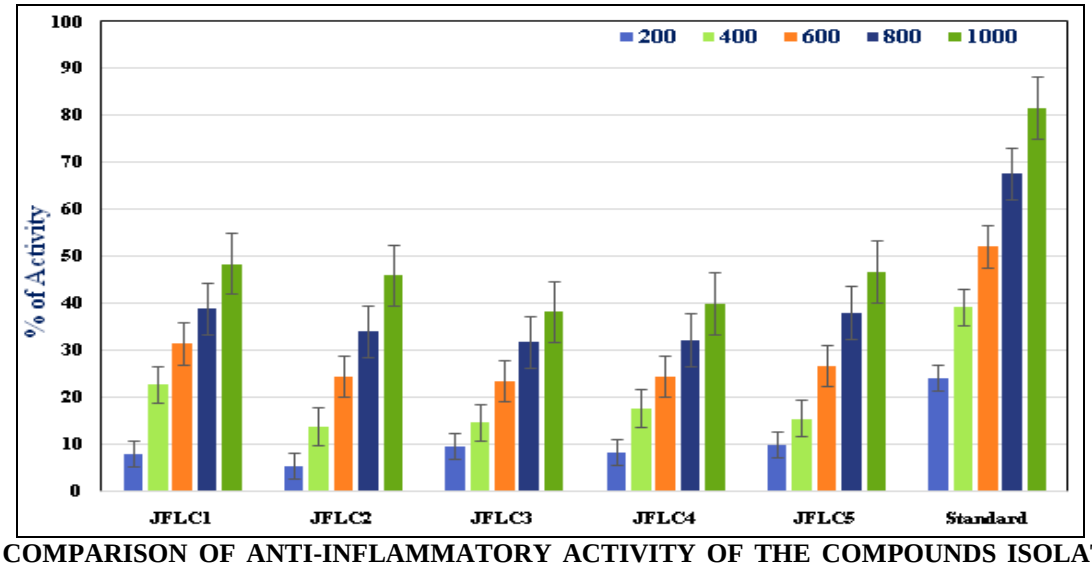

CHART 3: COMPARISON OF ANTI-INFLAMMATORY ACTIVITY OF THE COMPOUNDS ISOLATED FROM THE LEAVES OF *JASMINUM FLEXILE*

CONCLUSION: This study validates the antibacterial and *in-vitro* anti-inflammatory potential of the leaf hydroethanol extract of *Jasminum flexile*, its column fractionates, and compounds isolated. The results indicate that the leaves of *Jasminum flexile* possess bioactive that may be further explored for the developing of new drugs. This study also validates the significant contribution of the leaves of *Jasminum flexile* in the anti-inflammatory and wound healing efficacy of the Ayurvedic *Jatyadithailam.*

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CONFLICTS OF INTEREST: The authors declare no Conflict of Interest

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