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In-vitro evaluation of preliminary anti-inflammatory and antimicrobial activities of Ratha taila (medicated oil) and development of a novel emulgel formulation from its ingredients

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ABSTRACT

Ratha Taila is a traditional Ayurvedic oil for skin disorders containing *Ixora coccinea, Croton aromaticus, Gossypium herbaceum, Cocos nucifera,* and *Sesamum indicum* oil. This study evaluated the anti-inflammatory and antimicrobial properties of Ratha Taila and developed a novel emulgel formulation to overcome oil-based delivery limitations. Aqueous extracts were prepared using traditional decoction methods. Anti-inflammatory activity was assessed using the egg albumin denaturation assay, while antimicrobial activity was evaluated against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* using disc diffusion and broth microdilution methods. Emulgel formulations (5%, 10%, and 20% w/w) were prepared using Tween 20 and Carbopol 940, with stability assessed over 46 days. Results showed that Ratha Taila and its ingredients, except *C. nucifera*, had significant preliminary anti-inflammatory and antimicrobial effects ($p < 0.05$). Ratha Taila exhibited the strongest activity against the tested microorganisms. The 20% emulgel formulation showed promising anti-inflammatory activity compared to diclofenac sodium gel and exhibited significant antimicrobial activity against selected microorganisms. *Cocos nucifera* demonstrated no significant anti-inflammatory or antimicrobial activity. In conclusion, Ratha Taila, its ingredients except *C. nucifera,* and formulated emulgel demonstrated preliminary antimicrobial and anti-inflammatory activities.

1. INTRODUCTION

Ayurveda has effectively utilized therapeutic oils [1], herbal remedies, and medicinal plants [2] over the ages to treat a variety of illnesses. The goal of Ayurveda treatment is to balance the mind, body, and spirit through a holistic approach. An Ayurvedic oil called Ratha Taila is used widely in Sri Lanka to treat a variety of skin disorders, especially Rathagaya, a condition analogous to atopic dermatitis, but there is limited scientific evidence about Ratha Taila [3]. Due to its diverse composition, Ratha Taila has been shown to have anti-inflammatory, anti-microbial, and skin-healing qualities, making it particularly advantageous for treating inflammatory skin conditions [3]. These properties of Ratha Taila are primarily attributed to the bioactive chemical components in its key ingredients.

The ingredients of Ratha Taila include ripe *Croton aromaticus* leaves, *Ixora coccinea* flower buds, *Cocos nucifera* leaves, leaves of *Gossypium herbaceum* and *Sesamum indicum* oil as the base oil. *I. coccinea* contains lupeol [4], ursolic acid [5], and oleanolic acid, which have been shown to inhibit pro-

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inflammatory cytokines and reduce swelling by modulating the NF-kB pathway [6], a key regulator of inflammation. Primary isolation studies confirm that *I. coccinea* flowers contain lupeol, ursolic acid, and oleanolic acid as major triterpenes [7], with ultrasound-assisted extraction achieving 35% yield of ursolic acid from flowers. Anti-inflammatory activities of lupeol are isolated from *I. coccinea* leaves and demonstrate significant activity [8]. Mechanistic evidence shows oleanolic acid down-modulates NF-κB both *in vitro* and *in vivo* [9], while ursolic acid inhibits NF-κB in macrophages and reduces colitis [10], and lupeol inhibits NF-κB, reducing cytokines in inflammatory models [11]. *Croton aromaticus* is rich in alkaloids, terpenoids, and flavonoids [12]. It inhibits prostaglandin synthesis and has an antibacterial effect against methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Primary studies isolated (-) hardwickiic acid from *C. aromaticus* roots, demonstrating bioactivity [13], with cyperenoic acid and hardwickiic acid showing insecticidal properties [14]. Antifungal activity of *C. aromaticus* leaf extracts against post-harvest pathogens has been confirmed [15]. Carbohydrates, saponins, steroids, and glycosides in *G. herbaceum* reduces vascular permeability and therefore suppress inflammation [16]. In addition, it has antifungal [17,18], antiviral [19], and antibacterial actions. Chemical composition analysis confirms *G. herbaceum* leaves contain major components, including linoleic acid (36.1%), vitamin E (7.15%), and caryophyllene (4.21%), with comprehensive antioxidant and antibacterial profiling [20]. Furthermore, Vitamin E and sesamin are also present in *S. indicum*, which are potent anti-inflammatory substances that have the ability to depress inflammation and reactive oxygen species (ROS) [19,21]. Sesame seeds contain lignans with mean levels of sesamin (~2.48 mg/g) and sesamolin (~1.72 mg/g) [22], while sesame oil demonstrates fatty acid composition with oleic acid (~39%) and linoleic acid (~44%) [23]. Tocopherols are abundant in sesame oil, with γ-tocopherol being predominant [24]. Anti-inflammatory activity of sesame oil and sesamin has been demonstrated *in vivo* using carrageenan models, showing reduced exudate and leukocyte migration [25].

Atopic dermatitis is a common, chronic condition that can have a significant, negative effect on an individual's quality of life. Skin diseases are common in developing countries and affect 20% of children and 1-3% of adults [22,26]. Although limited, modern scientific research has been conducted to evaluate the effectiveness of the oil in treating these conditions [27]. Traditional herbal oils like Ratha Taila face limitations, including poor patient compliance due to their greasy texture and limited skin penetration [29]. Emulgel formulations address these challenges by offering a nongreasy texture, enhanced skin penetration, improved stability, and sustained release properties [29]. This pharmaceutical approach eliminates compliance issues while maintaining therapeutic efficacy through improved bioavailability and professional presentation [30]. In addition, emulgels present a more pharmaceutical-grade appearance that increases patient confidence and clinical acceptability compared to traditional oil preparations. Emulgels possess the properties of both emulsion and gel systems, and provide better stability along with a nongreasy feel, along with increased action of permeability of active ingredients [31]. By improving the spreadability, applicability, and sustained release of therapeutic agents, this new method of formulation represents a patient-friendlier alternative to traditional oil-based options [32,33].

Although Ratha Taila is of historical importance and is currently in use, its therapeutic effects are not well supported by scientific evidence [33]. This study attempts to bridge the old practices of Ayurveda with modern pharmaceutical approaches for the increasing demand of herbal drugs and alternative medicine. This study provides the first scientific evaluation of traditional Ratha Taila's complete formulation for anti-inflammatory and antimicrobial activities. The novelty lies in validating the traditional combination and developing a modern emulgel formulation to address oil-based delivery limitations. This approach bridges traditional Ayurvedic knowledge with contemporary pharmaceutical innovation, improving patient compliance while maintaining therapeutic principles. These preliminary findings establish a foundation for future cell-based assays, *in vivo* validation, and clinical studies.

The main hypothesis of this study is that Ratha Taila has measurable anti-inflammatory and anti-microbial qualities, which can be verified using *in-vitro* testing methods. Furthermore, it is anticipated that the removal of the greasy feeling associated with conventional oils by emulsifying Ratha Taila into a modified emulgel formulation can increase its bioavailability, therapeutic efficacy, and patient compliance [34]. The present study aims to fill the above gap by conducting a comprehensive *in-vitro* evaluation of preliminary anti-inflammatory and antimicrobial properties of Ratha Taila and its ingredients, and to investigate its potential in a modified dosage form, emulgel, to enhance its therapeutic effectiveness.

2. MATERIALS AND METHODS

2.1 Plant collection and authentication

Fresh plant materials of *C. aromaticus*, *I. coccinea*, and *C. nucifera* were collected from Rathnapura, Sabaragamuwa Province, Sri Lanka and *G. herbaceum* from Embilipitiya, Sabaragamuwa Province, Sri Lanka in February 2023, following approval from the Faculty of Indigenous Medicine, University of Colombo. Plant collection was conducted with appropriate institutional permissions and following sustainable harvesting practices. No endangered species were involved in this study. Plant specimens were authenticated at the Bandaranaike Memorial Ayurvedic Research Institute, Nawinna, Maharagama, Sri Lanka, and voucher specimens were deposited with accession numbers: *C. aromaticus* (4026), *I. coccinea* (4027), *G. herbaceum* (4028), and *C. nucifera* (4029) [35].

2.2 Preparation of aqueous plant extracts

Authenticated plant materials were processed using traditional Ayurvedic decoction methods (Kwatha) to preserve bioactive compounds and therapeutic integrity [36]. Plant materials were cleaned, shade-dried for 48 hours, and coarsely powdered using a mechanical grinder. Standardized decoctions were prepared by boiling 100 g of plant material with 400 ml of distilled water until volume reduced to 100

ml (1:4:1 ratio). Extracts were filtered through Whatman No. 1 filter paper, concentrated under reduced pressure at 60°C using a rotary evaporator (Heidolph Laborota 4000, Germany), and freeze-dried using lyophilizer (Christ Alpha 2-4 LD plus, Germany) to obtain standardized powder extracts with a moisture content <5% [37]. Extract yields were: *C. aromaticus* (12.8%), *I. coccinea* (14.2%), *G. herbaceum* (11.5%), and *C. nucifera* (9.7%) from dried plant material to lyophilized powder.

2.3 Preparation of Ratha taila according to the ayurveda formula

Ratha Taila was prepared following authentic Ayurvedic Pharmacopoeia guidelines to ensure therapeutic validity and traditional compliance [38]. Fresh plant materials were processed according to classical methodology: *C. aromaticus* leaves, *G. herbaceum* leaves, and *I. coccinea* flower buds were separately ground with distilled water using a traditional stone grinder to obtain 240 ml each of standardized plant juices. These were combined with 240 ml freshly extracted *C. nucifera* leaf juice and 240 ml cold-pressed sesame oil (*S. indicum*). The mixture was subjected to controlled solar heating (Taila Paka method) until an appropriate Paaka Avastha (oil maturation stage) was achieved, as determined by traditional organoleptic criteria including foam formation, sound production, and characteristic aroma development [39].

2.4 Investigation of the *in vitro* anti-inflammatory activity of ratha taila and its ingredients

The *in vitro* preliminary anti-inflammatory activity of Ratha Taila and its individual ingredients (ripe leaves of *C. aromaticus*, flower buds of *I. coccinea,* leaves of *G. herbaceum*, and leaves of *C. nucifera*) were evaluated using the egg albumin denaturation assay [40].

Test samples were prepared in concentration series (39.06, 78.12, 156.25, 312.5, 625, 1250, 2500, and 5000 µg/ml) using serial dilution in phosphate buffer (pH 6.4). Reaction mixtures (5 ml total volume) contained: 0.2 ml fresh egg albumin (hen's egg), 2.8 ml phosphate buffer (pH 6.4), and 2.0 ml test sample at specified concentrations. Diclofenac sodium (pharmaceutical grade) served as a positive control using an identical concentration range, while distilled water functioned as a negative control [41].

Reaction mixtures were incubated at 37°C for 15 minutes in a temperature-controlled water bath, subsequently heated to 70°C for 5 minutes with continuous gentle agitation, then rapidly cooled to room temperature using an ice bath. Turbidity was measured at 660 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan) against a blank containing all components except albumin [41]. Percentage inhibition of protein denaturation was calculated using the formula:

Percentage inhibition = A control – A sample / A control*100.

The IC_{50} value was determined by plotting the percentage inhibition against the log concentration of the respective sample [42].

2.5 Investigation of the anti-microbial activity of Ratha taila and its ingredients

2.5.1. Agar disc diffusion assay

Antimicrobial activity was assessed against clinically relevant skin pathogens, including *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853), and *C. albicans* (ATCC 10231), using the agar disc diffusion method, the anti-microbial properties of Ratha Taila and its component ingredients *(*ripe leaves of *C. aromaticus*, flower buds of *I. coccinea,* leaves of *G. herbaceum*, leaves of *C. nucifera,* and oil of *S. indicum*) were evaluated [43].

Mueller-Hinton agar plates were inoculated with standardized microbial suspensions (1.5×10^8 CFU/ml for bacteria, 1.5×10^6 CFU/ml for fungi). Sterile 6 mm filter paper discs were impregnated with test samples and placed on inoculated agar surfaces. Gentamicin (10 µg/disc) served as a positive control for bacteria, fluconazole (25 µg/disc) for fungi, and Dimethyl sulfoxide (DMSO) as a negative control. Plates were incubated at 37°C for 24 hours, and inhibition zone diameters were measured using digital calipers [44–46].

2.5.2. *Broth microdilution method*

Half-maximal inhibitory concentrations (IC_{50}) were determined using the broth microdilution technique in sterile 96-well microplates [26]. Test samples were prepared in DMSO (<1% final concentration) and serially diluted in Mueller–Hinton broth media to achieve a concentration range of 5,000–39.06 µg/ml. Each well received 100 µl sterile broth medium, followed by 100 µl test sample dilutions. Standardized microbial inocula (5 µl of 1.5×10^5 CFU/ml suspension) of *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853), and *C. albicans* (ATCC 10231) were added to test wells, with growth control wells receiving inoculum without test samples and sterility control wells containing only broth and test samples. Plates were incubated at 37°C for 18–20 hours with gentle agitation every 6 hours. Absorbance measurements were obtained using an ELISA microplate reader. The percentage inhibition was calculated [47].

2.6. Development of topical formulation

2.6.1. Preparation of primary emulsion

The aqueous phase of the emulsion was prepared by dissolving the freeze-dried extracts of ripe leaves of *C. aromaticus*, flower buds of *I. coccinea,* leaves of *G. herbaceum*, and leaves of *C. nucifera* in a 1:1:1:1 ratio. Sesame oil was used as the oil phase. Tween 20 was used as the gum phase. The oil phase was slowly added to the gum phase with continuous stirring to achieve proper dispersion. The aqueous phase was then added dropwise to the oil phase, and the emulsion was formed by vigorous stirring. The primary emulsion was allowed to stabilize and rest for a specified period [48].

2.6.2. Preparation of gel formulation

A total of 1 gram of Carbopol 940 [49] powder was slowly sprinkled into the distilled water while continuously stirring to avoid clumping. The pH of the gel was adjusted using

triethanolamine (TEA) based on the specific requirements. After pH adjustment, the gel mixture was stirred gently or subjected to gentle agitation to ensure uniform dispersion of the Carbopol 940 and to promote gel formation [50].

2.6.3. Preparation of emulgel

Emulgel formulations containing 5%, 10%, and 20% (w/w) of combined plant extracts were prepared by incorporating the primary emulsion into the gel base using gentle mechanical stirring until homogeneous consistency was achieved. Formulations were stored in airtight containers at room temperature pending evaluation [51].

2.7. Stability test for formulated emulgel

2.7.1. Visual observation

Comprehensive stability evaluation was conducted over 46 days under controlled storage conditions, including refrigeration (8°C ± 2°C), room temperature (25°C ± 2°C), and accelerated conditions (40°C ± 2°C). Physical parameters assessed included appearance, color, odor, phase separation, pH stability, and rheological properties.

2.7.2. Centrifuge testing

Emulgel samples (5 g) were subjected to centrifugation at 3000 rpm for 20 minutes using a refrigerated centrifuge (Eppendorf 5810R, Germany) to evaluate phase separation tendency under mechanical stress.

2.7.3. Freeze-thaw test

The emulgel formulations of 5%, 10%, and 20% (w/w) were subjected to a three freeze-thaw procedure, with each cycle consisting of a 24-hour freeze at −20°C followed by a 24-hour thaw at room temperature. Physical changes, such as phase separation or texture alterations, were monitored to assess the stability of the emulgel samples under freeze-thaw conditions.

2.7.4. Checking the pH

The pH of the emulgel formulations of 5%, 10%, and 20% (w/w) was checked using a pH meter.

2.7.5. Spreadability assessment

Gel spreadability was determined using the standardized cone and plate method. One gram of formulation was placed between two glass plates (10 cm diameter), and a 100 g weight was applied for 5 minutes. Spread diameter was measured using digital calipers, with spreadability calculated as follows:

$$\text{Spreadability (cm)} = (\pi \times d^2)/4$$, where d represents the mean diameter of the spread area.

2.8. Investigation of the *in vitro* anti-inflammatory activity of emulgel

The preliminary anti-inflammatory activity of the 20 % emulgel and diclofenac sodium gel was evaluated using the egg albumin denaturation assay [41]. Diclofenac sodium gel was used as the positive control. A 5 ml reaction mixture was prepared by adding 0.2 ml of egg albumin, 2.8 ml of phosphate buffer (pH 6.4), 2.0 ml of emulgel (at different concentrations ranging from 39.06 µg/ml to 5,000 µg/ml), and diclofenac sodium gel (at different concentrations ranging from 39.06 µg/ml to 5,000 µg/ml) used as positive control. The absorbance of the samples was measured after incubation and heating, as described at 2.4. The percentage inhibition of protein denaturation was calculated, and the IC_{50} value for emulgel was determined.

2.9. Investigation of the anti-microbial activity of emulgel

The antimicrobial activity of the 20 % emulgel was evaluated using the well diffusion method. A sterile cork borer was used to create wells in the agar. MHA plates were inoculated with the test microorganisms, including *S. aureus, P. aeruginosa,* and *C. albicans*. 20 µl of emulgel (prepared in 10% DMSO) was added into each well. Positive controls (gentamicin for bacteria and fluconazole for fungi) were included, with DMSO as the negative control. The antimicrobial activity was evaluated by measuring the zones of inhibition following 24 hours of incubation at 37°.

The IC_{50} values of the emulgel were determined using the broth microdilution method, as described in 2.5.2.

2.10. Statistical analysis

All experiments were performed in triplicate ($n = 3$) with results presented as mean ± standard error of mean (SEM). IC_{50} values were calculated using nonlinear regression analysis with four-parameter logistic curve fitting in GraphPad Prism version 9.0 software. Statistical significance between multiple groups was evaluated using one-way analysis of variance (ANOVA) followed by Tukey's honest significant difference (HSD) post hoc test. For two-group comparisons, a t-test was employed. *P*-values < 0.05 were considered statistically significant, with exact *p*-values reported where applicable. Coefficient of determination (R^2) values were calculated to assess goodness of fit for dose-response relationships. Effect sizes were calculated using Cohen's d for practical significance assessment.

3. RESULTS

3.1. Investigation of the *in vitro* anti-inflammatory activity of ratha taila and, its ingredients

One-way ANOVA revealed significant differences between treatment groups ($F_{7,19} = 89.34$, $p < 0.05$), with treatment explaining 97.5% of observed variance ($\eta^2 = 0.975$). Ratha Taila demonstrated significant anti-inflammatory activity with a statistically higher IC_{50} value compared to diclofenac sodium ($p < 0.05$). Among individual components, the anti-inflammatory potency ranking was *C. aromaticus* > *I. coccinea* > *G. herbaceum* > *S. indicum*. Tukey's post hoc analysis revealed that Ratha Taila was significantly more potent than *G. herbaceum* and *S. indicum* ($p < 0.05$) but showed no significant difference from *C. aromaticus* and *I. coccinea* ($p > 0.05$). *Sesamum indicum* exhibited significantly weaker activity compared to all other samples ($p < 0.05$). *Cocos nucifera* demonstrated no significant anti-inflammatory activity, with percentage inhibition not

exceeding 20% at the maximum tested concentration (Table 1). All active samples demonstrated dose-response relationships with correlation coefficients exceeding 0.99 ($p < 0.05$), confirming concentration-dependent activity (Fig. 1).

3.2. Investigation of the anti-microbial activity of Ratha taila and its ingredients

3.2.1. Agar disc diffusion assay

Two-way ANOVA revealed significant main effects of treatment ($F_{5,36}$= 156.78, $p < 0.05$), microorganism type ($F_{2,36}$= 67.45, $p < 0.05$), and significant treatment × microorganism interaction ($F_{10,36}$ = 23.12, $p < 0.05$). Ratha Taila produced the largest inhibition zones against all tested pathogens, with activity ranking: *S. aureus* > *P. aeruginosa* > *C. albicans*. Among individual components, *I. coccinea* demonstrated the most consistent antimicrobial activity across all organisms with statistically significant zones compared to negative controls ($p < 0.05$). C. *aromaticus* and *G. herbaceum* showed moderate activity against bacterial pathogens but limited antifungal effects. *C. nucifera* showed minimal activity with inhibition zones not significantly different from negative controls ($p > 0.05$) (Table 2, Fig. 2).

3.2.2. Broth microdilution method

Ratha Taila demonstrated significant antimicrobial activity against all tested pathogens ($p < 0.05$ vs. negative controls), with the highest potency against *P. aeruginosa. I. coccinea* exhibited significantly lower IC_{50} values than other extracts across all organisms ($p < 0.05$), establishing it as the primary antimicrobial contributor. The antimicrobial potency hierarchy was *I. coccinea* > *Ratha Taila* > *C. aromaticus* > *G. herbaceum* > *S. indicum* >> *C. nucifera. C. nucifera* showed no significant antimicrobial activity compared to negative controls ($p > 0.05$) (Table 3).

Table 1. Half-maximal inhibitory concentration (IC_{50}) values, coefficient of determination (R^2), SEM, and 95% confidence intervals for anti-inflammatory activity of Ratha Taila, its individual components, and reference controls assessed using egg albumin denaturation assay.

Sample	IC_{50}(µg/ml)	R^2 Value	SEM	95% CI (IC_{50})
Diclofenac Sodium	538.30	0.99	±2.52	512.30 - 564.30
Ratha Taila	571.60	0.99	±3.11	530.10 - 613.10
I. coccinea	682.70	0.99	±4.11	640.00- 725.30
C. aromaticus	648.50	0.99	±3.77	610.10- 686.90
G. herbaceum	727.40	0.99	±4.22	687.20- 767.60
S. indicum	910.20	0.99	±5.16	870.50- 950.30
Emulgel	511.60	0.99	±3.22	520.40- 581.20
Diclofenac Sodium gel	538.00	0.99	±2.65	512.40- 563.60

Data represent mean ± SEM of three independent experiments ($n = 3$). IC_{50} values were determined using four-parameter logistic nonlinear regression analysis. R^2 = coefficient of determination; CI = confidence interval. All correlations were statistically significant ($p < 0.05$).

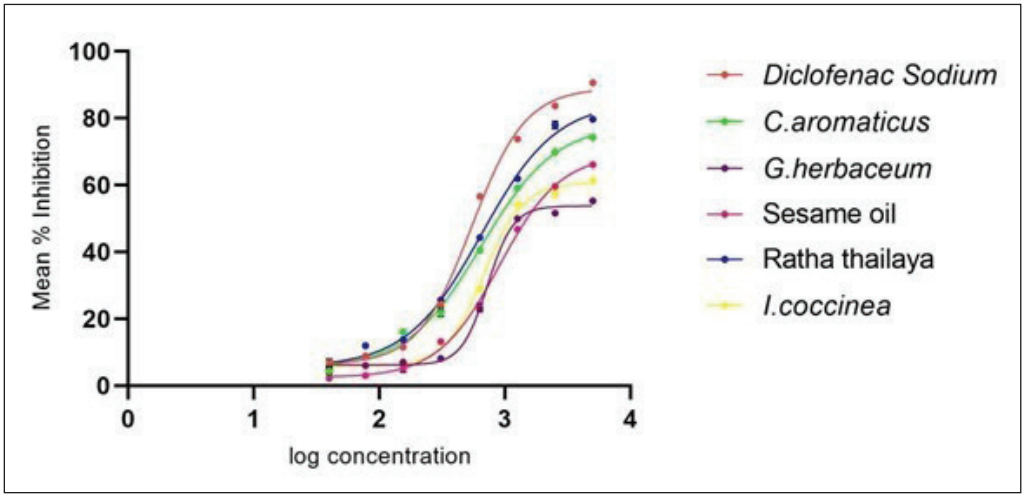

Figure 1. Dose-response curves showing anti-inflammatory activity of Ratha Taila and individual components compared to diclofenac sodium using egg albumin denaturation assay. X-axis: Log concentration (µg/mL); Y-axis: Percentage inhibition of protein denaturation (%). Data represent mean ± SEM of three independent experiments (n=3). Curves fitted using four-parameter logistic nonlinear regression ($R^2 > 0.99$ for all samples).

Table 2. Mean diameter of zone of inhibition (mm) for Ratha Taila and its ingredients against *S.aureus, P. aeruginosa,* and *C. albicans.* Values are expressed as mean ± SD.

Sample	*S. aureus*		*P. aeruginosa*		*C. albicans*	
	Sample(mm)±SD	Positive control(mm)±SD	Sample(mm)±SD	Positive control(mm)±SD	Sample(mm)±SD	Positive control(mm)±SD
Ratha Taila	32.7 ± 2.1	36.3 ± 1.5	26.1 ± 1.7	33.0 ± 1.7	15.7 ± 2.0	26.3 ± 1.5
I. coccinea	31.7 ± 2.9	34.7 ± 1.5	22.7 ± 2.5	34.0 ± 1.1	20.0 ± 2.3	24.3 ± 1.5
C. aromaticus	28.0 ± 1.0	34.7 ± 1.5	14.3 ± 4.0	34.0 ± 1.1	11.6 ± 1.5	24.3 ± 1.5
G. herbaceum	15.7 ± 1.5	32.7 ± 2.5	14.3 ± 4.0	32.7 ± 2.5	12.3 ± 2.5	24.3 ± 1.5
S. indicum	12.3 ± 2.5	32.7 ± 2.5	18.3 ± 2.9	32.7 ± 2.5	12.3 ± 2.5	24.3 ± 1.5
Emulgel	13.0 ± 1.0	21.0 ± 1.0	30.0 ± 1.0	36.7 ± 1.5	16.7 ± 1.5	19.3 ± 2.1

Data represent mean ± SD of three independent experiments ($n = 3$). Test samples were applied at 10 mg/ml concentration on 6 mm diameter discs. Positive controls: Gentamicin (10 µg/disc) for bacteria, Fluconazole (25 µg/disc) for fungi. Zone diameters include disc diameter. Inhibition zones <6 mm were considered inactive.

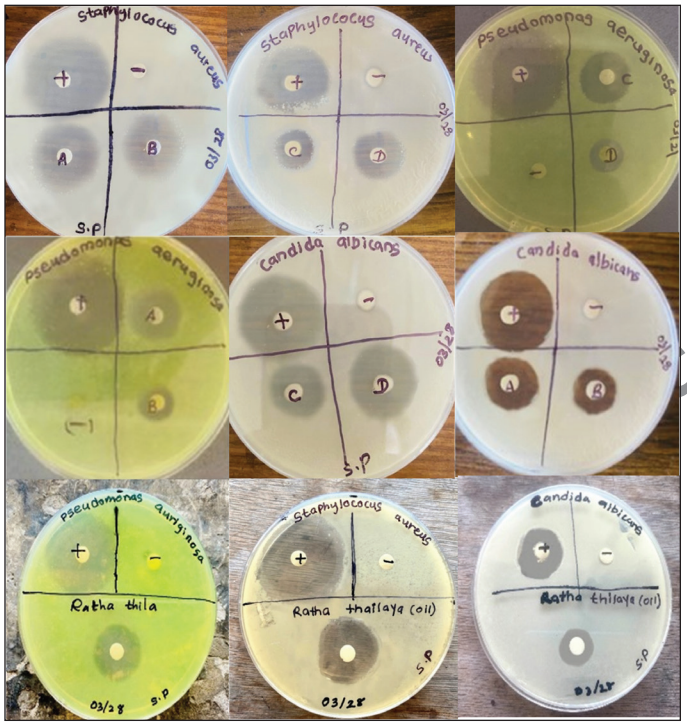

Figure 2. - Zone of inhibition of aqueous extracts of *I. coccinea* (A), *C. aromaticus* (B), *S. indicum* (C), *G. herbaceum* (D), and Ratha Taila disc diffusion assay against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. Test samples applied at 10 mg/mL concentration on 6 mm diameter discs. Positive controls: Gentamicin (10 µg/disc) for bacteria, Fluconazole (25 µg/disc) for fungi; Negative control: DMSO (10% v/v). Plates incubated at 37°C for 24 hours.

3.3. Stability tests for formulated emulgel

The 5% w/w formulation (E1) maintained excellent physical stability across all temperature conditions throughout the study period**.** Chi-square analysis revealed no significant changes in appearance, color, or odor under any storage conditions. The formulation retained homogeneous consistency with no visible phase separation or texture alterations. The 10% w/w formulation (E2) exhibited major stability problems, particularly under elevated temperature conditions. By day 16, visible color changes and appearance alterations were observed at 40°C, progressing to complete instability by day 46. This instability of the 10% formulation represents a significant limitation for practical application. The 20% w/w formulation (E3) demonstrated superior stability characteristics across all tested conditions. Stability assessment revealed 100% sample retention without deterioration throughout the 46-day study period under all storage conditions tested. pH values for 5% and 20% formulations remained within skin-compatible ranges (pH 6.19-6.24), while spreadability analysis revealed superior application characteristics for 5% and 20% formulations compared to the 10% formulation. Spreadability findings reveal that the spreading of 5% and 20% formulations is better than that of the 10% formulation.

The stability characteristics of all three formulations (5%, 10%, and 20% w/w) are summarized in Table 4.

3.4. Investigation of the *in vitro* anti-inflammatory activity of emulgel

The 20% w/w emulgel formulation demonstrated preliminary anti-inflammatory activity that showed no significant difference from traditional Ratha Taila (unpaired t-test, $p > 0.05$), indicating maintained therapeutic equivalence. However, comparison with diclofenac sodium gel revealed a statistically significant difference ($p < 0.05$), with the emulgel showing statistically lower potency.

Dose-response analysis revealed excellent correlation between concentration and inhibitory activity ($R^2 = 0.997$, $p < 0.05$), confirming a reliable and predictable therapeutic response (Fig. 3).

3.5. Investigation of the anti-microbial activity of emulgel

3.5.1. Well diffusion assay

The 20% emulgel formulation demonstrated significant antimicrobial activity against all tested organisms ($p < 0.05$ vs. negative controls). One-way ANOVA revealed significant differences between emulgel and negative controls ($p < 0.05$) but significantly smaller zones compared to positive controls ($p < 0.05$). Activity ranking was: *P. aeruginosa* > *C. albicans* > *S. aureus*. The emulgel's activity against *P. aeruginosa*

Table 3. Half-maximal inhibitory concentration (IC_{50}) values and coefficient of determination (R^2) for antimicrobial activity of Ratha Taila, its individual components, and emulgel formulation determined by broth microdilution method.

Sample	*S. aureus* IC_{50} ± SEM (µg/ml)	*P. aeruginosa* IC_{50} ± SEM (µg/ml)	*C. albicans* IC_{50} ± SEM (µg/ml)	R^2 Value
Ratha Taila	240.5 ± 3.1	217.3 ± 2.1	250.1 ± 2.3	0.99
I. Coccinea	149.4 ± 3.0	246.2 ± 3.0	226.7 ± 2.9	0.99
C. aromaticus	309.4 ± 2.7	460.0 ± 3.3	297.5 ± 3.1	0.99
G. herbaceum	556.1 ± 4.2	654.5 ± 4.1	339.6 ± 3.5	0.99
S. indicum	528.0 ± 4.1	268.8 ± 3.6	311.8 ± 3.2	0.99
C. nucifera	1200.0 ± 5.2	1160.2 ± 5.4	950.3 ± 5.5	0.98
Emulgel	550.8 ± 3.4	464.5 ± 2.7	344.5± 3.6	0.99

Data represent mean ± SEM of three independent experiments ($n = 3$). IC_{50} values were calculated using four-parameter logistic nonlinear regression analysis from eight-point concentration curves (5000–39.06 µg/ml). R^2 = coefficient of determination. Values >5,000 µg/ml indicate no significant antimicrobial activity within tested concentration range.

Table 4. Comprehensive stability characteristics of emulgel formulations over 46-day study period.

Stability parameter	5% Emulgel (E1)	10% Emulgel (E2)	20% Emulgel (E3)
Visual stability (8°C)	No changes observed	Slight color change after day 30	No changes observed
Visual stability (25°C)	Excellent stability	Color changes after day 16	Excellent stability
Visual stability (40°C)	Good stability	Complete deterioration by day 46	Excellent stability
Phase separation (centrifuge test)	No separation	Separation observed	No separation
Freeze-thaw stability (3 cycles)	Stable, no separation	Stable, no separation	Stable, no separation
pH (mean ± SD)	6.19 ± 0.05	4.98 ± 0.08	6.24 ± 0.06
Spreadability diameter (cm ± SD)	2.37 ± 0.12	1.9 ± 0.15	2.5 ± 0.10

Data represent mean ± SD of three independent measurements ($n = 3$). Centrifuge test: 3,000 rpm for 20 minutes. Freeze-thaw test: 3 cycles of −20°C (24h) followed by room temperature (24h). pH measured using calibrated pH meter. Spreadability determined using standard cone and plate method with 100g weight for 5 minutes.

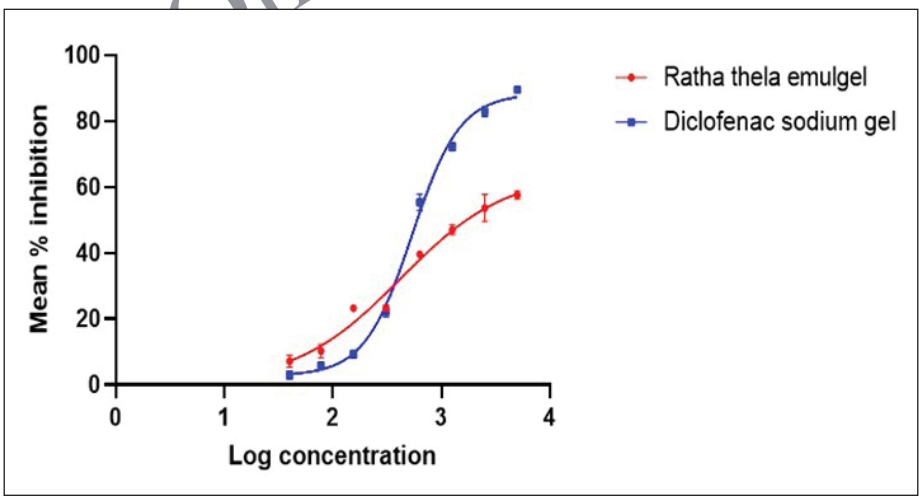

Figure 3. Comparative anti-inflammatory activity of Ratha Taila emulgel (20% w/w) and diclofenac sodium gel assessed by egg albumin denaturation assay. X-axis: Log concentration (µg/mL); Y-axis: Percentage inhibition of protein denaturation (%). Data represent mean ± SEM of three independent experiments ($n = 3$). Statistical analysis by unpaired Student's *t*-test.

approached positive control efficacy (no significant difference, $p > 0.05$), while showing significant but moderate activity against *S. aureus and C. albicans* compared to respective controls ($p < 0.05$) (Fig. 4).

3.5.2. Broth microdilution method

Broth microdilution analysis confirmed antimicrobial activity with measurable IC_{50} values against all tested microorganisms. The emulgel maintained antimicrobial activity

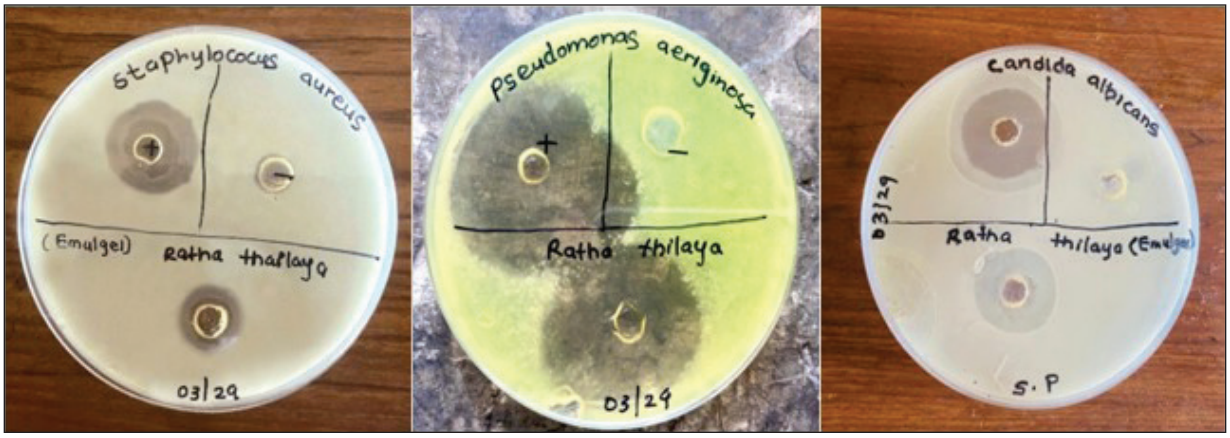

Figure 4. Antimicrobial activity of Ratha Taila emulgel (20% w/w) assessed by well diffusion assay. Representative photographs showing inhibition zones against (A*) Staphylococcus aureus*, (B) *Pseudomonas aeruginosa*, and (C) *Candida albicans*. Emulgel applied as 20 µl per well (6 mm diameter). Positive controls: Gentamicin gel for bacteria, Fluconazole gel for fungi.

against the tested organisms with activity hierarchy: *C. albicans* > *P. aeruginosa* > *S. aureus*, demonstrating retained bioactivity suitable for caused by these pathogens ($p < 0.05$ vs. negative controls for all organisms).

4. DISCUSSION

This study assessed the preliminary anti-inflammatory and antimicrobial properties of Ratha Taila and its components, together with a newly formulated emulgel. This study aimed to improve the application and bioavailability of traditional Ratha Taila while evaluating its effectiveness in a modern formulation for enhanced patient compliance, which was not previously attempted through a scientific study.

Plant extracts were prepared by following the decoction method described in the traditional Ayurveda. It can be successfully used for the extraction of bioactive compounds with preservation of the healing potential [52,53]. The egg albumin denaturation assay was employed to evaluate the preliminary anti-inflammatory activity of Ratha Taila and its constituents. This assay has been widely recognized as a screening method for anti-inflammatory activity and was selected for its simplicity, reproducibility, and cost-effective nature. However, this method provides only preliminary screening data. Comprehensive mechanistic studies, including cell-based assays such as cytokine inhibition and NF-κB reporter assays, along with *in vivo* validation, are necessary to establish definitive anti-inflammatory efficacy [54,55]. This method has been extensively validated in recent literature, including comprehensive method reviews [41] and large-scale screening studies of medicinal plants. The IC_{50} of Ratha Taila was 571.6 µg/ml, which is statistically significantly higher than that of diclofenac sodium (538.3 µg/ml), indicating lower efficacy in reducing inflammation. Anti-inflammatory activity was evaluated by ANOVA and the Tukey post hoc test. Ratha Taila exerted an anti-inflammatory effect with statistically lower potency than diclofenac sodium ($p < 0.05$)**.** Further investigations are needed to explore the underlying mechanisms contributing to these differences.

Statistical analysis revealed that while Ratha Taila and its emulgel formulation demonstrated significant anti-inflammatory activity, they exhibited statistically lower potency than diclofenac sodium ($p < 0.05$). This difference may be clinically acceptable given the natural origin and potentially superior safety profile of plant-based formulations. The IC_{50} values of 571.6 µg/ml (Ratha Taila) and 511.6 µg/ml (emulgel) compared to 538.3 µg/ml (diclofenac) [56] represent promising activity that could be optimized through formulation modifications and standardization. The improved activity of the emulgel compared to traditional Ratha Taila suggests that modern pharmaceutical approaches can enhance traditional formulations without compromising their therapeutic principles.

Interestingly, the individual ingredients exhibited varying levels of anti-inflammatory potency. *C. aromaticus* exhibited the lowest IC_{50} value (648.2 µg/ml) among the constituent components, signifying the most potent anti-inflammatory activity. This finding is unprecedented, as no prior research has evaluated the anti-inflammatory properties of *C. aromaticus***.** While limited studies exist on *C. aromaticus* specifically, research on related Croton species supports anti-inflammatory potential through bioactive compounds [57]. Prior studies indicate that Croton species, containing chemicals such as alkaloids, terpenoids, and flavonoids, exhibit bioactive qualities with anti-inflammatory effects [58]. *I. coccinea* (IC_{50} = 682.7 µg/ml) and *G. herbaceum* (IC_{50} = 727.4 µg/ml) had moderate anti-inflammatory action, while *S. inducum* showed the least efficacy (IC_{50} = 910.2 µg/ml); its high IC_{50} value may be attributed to its primary role as an emollient [59] rather than as a direct anti-inflammatory agent. However, literature confirms that sesame oil exhibits anti-inflammatory and antinociceptive effects *in vivo* through cytokine down-regulation mechanisms involving IL-6 and IL-1 [24]. *C. nucifera* (Coconut), traditionally employed in Ayurvedic formulations for its moisturizing [60,61] and healing attributes, did not exhibit significant anti-inflammatory activity in this investigation ($p > 0.05$). This finding is consistent with literature demonstrating that while coconut combined with other components like wheatgrass can show significant albumin-denaturation inhibition, crude coconut extracts alone typically demonstrate minimal anti-inflammatory effects [62]. Previous studies have demonstrated

that *I. coccinea* contains substances such as ursolic acid and flavonoids have significant anti-inflammatory properties [63]. The modified emulgel formulation of Ratha Taila exhibited improved anti-inflammatory efficacy, with an IC_{50} of 511.6 µg/ml, in comparison with traditional Ratha Taila, which had an IC_{50} of 571.6 µg/ml. The improvement in activity could be due to the emulgel's ability to increase the solubility and penetration of bioactive chemicals into the skin, as previously evidenced in emulgel formulations designed for optimizing the topical administration of active components [64]. This finding is consistent with literature demonstrating that while coconut combined with other components like wheatgrass can show significant albumin-denaturation inhibition, crude coconut extracts alone typically demonstrate minimal anti-inflammatory effects. However, the emulgel still showed statistically significantly lower potency compared to diclofenac sodium gel ($p < 0.05$). Additional optimization regarding formulation and concentration is required to achieve efficacy comparable to diclofenac sodium gel. The advantages of Ratha Taila emulgel consist in its natural formulation, offering a safer alternative to synthetic medications, particularly for prolonged usage in the treatment of inflammatory skin conditions.

The antimicrobial activity of Ratha Taila and its constituent components was assessed using two methods: the agar disc diffusion assay and the broth microdilution technique. These techniques are commonly applied to the investigation of the antimicrobial efficacy of plant-based preparations and herbal products [65]. The tested organisms were *S. aureus, P. aeruginosa,* and *C. albicans,* which are common bacterial and fungal pathogens that cause a variety of skin conditions [66]. However, to establish comprehensive antimicrobial claims, testing against a broader panel of pathogens, including additional Gram-positive and Gram-negative bacteria as well as dermatophytes, would be necessary. The results indicate that Ratha Taila has antimicrobial activity against the tested organisms**, e**specially on Gram-positive bacteria like *S. aureus.* The activity observed could be due to the bioactive substances contained in the ingredients of Ratha Taila, such as flavonoids, alkaloids, and terpenoids, which showed the presence of antimicrobial activity in previous studies [33]. The antifungal potential in *C. albicans* was less compared to the positive control, Fluconazole.

The Broth Microdilution Method was used to determine the MIC and IC_{50} values of Ratha Taila and its components, providing a quantitative assessment of their antimicrobial activity [67]. The IC_{50} values for Ratha Taila were 240.5 µg/ml for *S. aureus*, 217.3 µg/ml for *P. aeruginosa*, and 250.1 µg/ml for *C. albicans*, indicating considerable antimicrobial activity, particularly against *P. aeruginosa*. Among the constituent ingredients, *I. coccinea* demonstrated the lowest IC_{50} values, indicating the highest antimicrobial efficacy, with 149.4 µg/ml against *S. aureus*, 246.2 µg/ml against *P. aeruginosa*, and 226.7 µg/ml against *C. albicans*. These findings align with previous studies that highlight the antimicrobial activity of *I. coccinea* against both bacterial and fungal pathogens [33,67]. These findings align excellently with published studies reporting I. coccinea leaf/flower/stem extracts showing zones of inhibition and MIC values of 0.78–3.125 mg/ml against *S. aureus*, *E. faecalis*, and *C. albicans* using identical disc diffusion and microdilution methodologies [65], validating both the results and methodology employed in this study. This study indicates that *C. aromaticus* exhibits significant antimicrobial efficacy, particularly against Gram-positive bacteria such as *S. aureus*. Previous research has also established its antifungal efficacy, especially against *C. albicans* supporting its antimicrobial potential [15].

However, *C. nucifera* did not show any significant antimicrobial activity ($p > 0.05$), as no zone of inhibition was observed, which is consistent with limited antimicrobial effects reported in earlier studies. This absence of activity indicates that *C. nucifera* contributes primarily to the emollient properties rather than the antimicrobial efficacy of Ratha Taila. While recent studies show that processed coconut derivatives like monolaurin demonstrate anti-*S. aureus* activity [68], this activity is attributed to processed compounds rather than crude leaf extracts [69].

The 20% (w/w) emulgel formulation of Ratha Taila showed stability during the 45-day storage period at different temperatures (8°C, 25°C, and 40°C). No phase separation was observed, in the 20% formulation, and the pH remained consistent. In contrast, the 10% formulation showed significant instability, including phase separation during centrifuge testing and visible deterioration by day 46 under accelerated conditions. This supports the findings of Peneva et al. (2018), who reported the stability of Carbopol 940-based emulgel formulations, which are widely used for topical applications. Recent literature on transforming medicinal oils into advanced gel formulations provides a comprehensive rationale for using Carbopol 940 and demonstrates the critical importance of optimal concentration selection for stability [70]. The pH of the emulgel composition was skin-friendly for dermatological application.

The emulgel development follows established precedent in Ayurvedic pharmaceutical innovation. Studies on Pinda Taila-loaded gel formulations have demonstrated improved patient acceptability and physicochemical stability compared to traditional oil formulations [64]. Research on converting other Ayurvedic oils like Prapaundarikadya Taila to semisolid dosage forms has shown enhanced spreadability, pH control, and clinical acceptability. Literature on emulgel formulations combining anti-inflammatory and antimicrobial activities, such as clove (*Eugenia caryophyllus*) emulgel using egg-albumin denaturation and antibacterial testing, provides strong precedent for the dual-activity approach employed. Studies on Jatyadi Thailam formulations have shown significant antimicrobial and anti-inflammatory effects, supporting the therapeutic potential of traditional Ayurvedic oil conversions [71].

The 46-day stability evaluation duration aligns with established protocols for herbal emulgel formulations. A recent study on Miswak oil emulgel conducted 45-day stability testing, reporting no significant changes in appearance, pH (6.77→6.64), viscosity, and drug content. This timeframe provides an adequate assessment of short-term stability characteristics essential for initial pharmaceutical development. The duration validates our approach for preliminary herbal emulgel evaluation [72]. The 5%, 10%, and 20% (w/w) concentrations were chosen based

on pharmaceutical principles and literature precedents. A 5% level was reported optimal for *Ocimum basilicum* emulgel, 10% represents a mid-range commonly tested in herbal gels, and 20% reflects the maximum therapeutic load with acceptable stability and compliance [73].

The present study provides valuable evidence on the anti-inflammatory and antimicrobial activities of and its emulgel formulation, Ratha Taila; however, clinical trials in humans are also necessary to validate their efficacy and safety. The *in-vitro* findings suggest the potential of Ratha Taila as a natural alternative to synthetic anti-inflammatory and antimicrobial agents, but these results must be validated in clinical settings to ensure their therapeutic relevance.

Future research should prioritize cell-based anti-inflammatory assays using human keratinocytes and immune cells to evaluate cytokine modulation and NF-κB pathway inhibition. Antimicrobial testing should be expanded to include broader pathogen panels, particularly dermatophytes relevant to skin infections. Safety evaluation through cytotoxicity assessment, skin irritation testing, and patch testing is required for topical pharmaceutical development. Formulation optimization should systematically evaluate different concentrations and gelling agents to enhance stability and efficacy. *In vivo* validation through animal studies followed by clinical trials will establish therapeutic efficacy, bioavailability, and safety profiles for clinical application.

5. CONCLUSION

Ratha Taila and its emulgel formulation demonstrated preliminary anti-inflammatory and antimicrobial activities against selected organisms. Among individual components, *I. coccinea* showed the highest antimicrobial efficacy, while *C. nucifera* demonstrated no significant activity. The 20% emulgel formulation exhibited improved anti-inflammatory activity compared to traditional oil and showed excellent stability. However, both formulations showed statistically lower potency than diclofenac sodium. This study provides preliminary scientific evidence supporting the traditional use of Ratha Taila and reports the successful development of its emulgel formulation. However, comprehensive evaluation through cell-based assays, expanded pathogen testing, safety assessments, and clinical trials is still required to establish its definitive therapeutic efficacy and safety profile.

6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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12. AI TOOL DISCLOSURE

Grammarly was used for grammar checking and English language editing to improve manuscript readability. No artificial intelligence tools were used for research design, data collection, analysis, interpretation, or scientific content creation.

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