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Anti-Inflammatory Activity Study of Baby Coconut Herbal Oil in Different Base Oils

Aneesha S*, Dr. Ankit Singh and Dr. Prasanth VV

Sri JJT University Rajasthan,

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Abstract

Background: Baby coconut (*Cocos nucifera*), a drupe rich in bioactive compounds, holds traditional promise for anti-inflammatory applications. This study evaluated herbal oils incorporating baby coconut ethyl acetate extracts in coconut, sesame, and mustard oil bases.

Methods: Herbal oils (F1: coconut; F2: sesame; F3: mustard) were prepared via 11-cycle infusion (12-15 days, 75-80°C) and standardized for acid value, saponification value, peroxide value, refractive index, and specific gravity. In vitro anti-inflammatory activity was assessed in LPS-stimulated RAW 264.7 macrophages using COX, LOX, MPO inhibition, and nitrite assays (25-100 µg/mL). Stability was monitored over 30 days.

Results: All formulations showed dose-dependent inhibition. Sesame oil-based F2 excelled overall: IC_{50} values—COX: 122.02 µg/mL, LOX: 94.02 µg/mL, MPO: 63.25 µg/mL; nitrite reduction at 100 µg/mL: 373.23 µg. Mustard oil (F3) led in COX (101.15 µg/mL) and nitrite (311.35 µg), coconut (F1) in stability. Sesame offered balanced potency via sesamin/sesamolin; coconut resisted oxidation best.

Conclusion: Sesame oil-based baby coconut herbal oil demonstrates superior multi-target anti-inflammatory efficacy, validating traditional uses for skin inflammations. Coconut base ensures stability, suggesting hybrid formulations. Findings support advancement to in vivo/clinical studies for natural cosmeceuticals.

Keywords: Baby coconut, *Cocos nucifera*, Herbal oil, Anti-Inflammatory activity, Sesame oil, Mustard oil, COX inhibition, LOX inhibition, Myeloperoxidase, Nitrite levels, RAW 264.7 macrophages, Pharmacognosy, In Vitro Assay, IC_{50}, stability study.

Introduction

Coconut is a drupe-like fruit with three layers of exocarp, mesocarp and endocarp and is cultivated in many countries. It is very much associated with Hindu rituals. Whole part is useful and having therapeutic properties. Coconut is used for its nourishment properties both internally and external cosmetic applications. The plant is well planted in warm, humid sunny climatic conditions with regular rainfall. Many studies show the therapeutic values and cosmetic values.

***Corresponding Author:**
Aneesha S
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This single-branched tree belongs to the family Arecaceae and originated from South East Asia an island between the Indian and Pacific oceans. Fruit is incorporated in making curries and the water and milk used as nutrition agent. Different parts of coconut fruit has been used for many health ailments like diarrhea, stomachaches, antipyretic, renal inflammation, amenorrhea, burns, menstrual cycle disorders, wound healing, oral contraception, diabetes, aphrodisiac, asthma, diuretic, gonorrhea, dysmenorrhea, veneral diseases, vaginal infections, malaria, and other antiviaral diaseases (EBC Lima 2015).

Externally, plant products are used for cosmetic purposes like hair growth, to reduce skin diseases like psoriasis, eczema, dermatitis, skin flaking, dryness, wrinkles, black spots, and sun tan (Babita Aggarwal, 2017). Coconut fruit contains nutrients like dietary fibers, fat, protein, vitamin B, vitamin C, calcium, magnesium, iron, phosphorus, potassium, and zinc.

Researchers found that the husk of coconut has the capacity to reduce pain. The husk fibre preparations are used in inflammatory conditions in Brazil. Husk also used for the white blood cell cancer therapies. Husk extract evaluated for the parasitic infections caused by gastrointestinal worms.

Coconut water contains amino acids and it can reduce free radical production and act as an effective antioxidant preparation. This may downgrade creatinine and urea levels, and calcium oxalate crystals. Virgin coconut oil has more antioxidant activity than that of commercial refined oil. It is effective increase bone density, decrease bone tissue and bone tissue separation etc. The potassium in the coconut may help to reduce cholesterol and thereby reducing the cardiac problems. Coconut root extract reduces depression and reduces seizure incidents. The endocarp extract examined for antimicrobial activity. The studies showed that it is effective against staphylococcus aureus, *Pseudomonas aeruginosa*, Escherichia coli, klebsiella pneumonia, Acinetobacter baumannii, etc. Oil and milk obtained from the endocarp of fruit effective in skin care preparations like cream and ointments. It effectively controls skin inflammations, ageing, tanning, wrinkles, and spot.

Materials and Methods

Collection and Preparation of Extracts

Fresh baby coconuts are collected from the palm lands. They are dried under the shade, crushed with hammers, and ground to a fine powder. The powder is sieved through sieve number 60. Ethyl acetate extracts were used for the anti-inflammatory studies.

Preparation of Medicated Oil

Preparation of decoctions

Collected sample materials washed properly and drain. Used a hand hammer to crush each small coconut and dried in an oven at 55 degree C. It was taken around 5 days to complete drying out. Used a mixer grinder to powder the sample. Around 25% of the fibers were still visible because they wouldn't undergo powdering due to their slightly hard nature. Finely powdered dried baby coconuts (24 gm) were mixed with four times the volume of previously collected rice water, mixed it well with an iron spatula.

Selection of base oils

Base oils were selected according to the absorptive capacity of the herb with the carrier oil and the previously known uses. Here selected coconut oil, sesame oil, and mustard oil were used for the herbal oil making procedure as they are known to have some qualities to reduce the inflammation.

Preparation of herbal oil

The various ingredients used for making herbal oil are sesame oil, coconut oil, and mustard oil, rice water, and fine herb powders. The oils were served as the base oil (300 ml). Rice Water added in an amount 4 to 8 times that of the oil. Herbal decoction was prepared in rice water, twice the amount of oil used. No preservatives used as the oil can be made by the user themselves whenever needed.

Components are heated gently in a traditional vessel like an iron kadai. The mixture is continuously stirred throughout the process to ensure even heat distribution. When the Herbal paste reaches a stage known as "mrudupaka" (rubbing the paste between the thumb and index finger). The herbal Paste became soft and

slightly sticky with moisture. This stage lasts only a few minutes during the entire procedure, so it's crucial to filter the oil carefully within this brief timeframe. The complete process of oil preparation spans about 12 to 15 days, during which the oil's temperature is consistently maintained between 75 and 80 degrees Celsius.
The medicated oils are intensely concentrated herbal oils. The repeated infusion of plant extracts and decoctions (11 times) enhances the oil's potency to 11 times greater than that of standard oil. The ingredients taken for the oil preparation entered in the table

Table 1: Formula for the preparation of herbal oil

Ingredients	F1	F2	F3
Drug (gm)	24	24	24
Coconut oil (ml)	300	-	-
Sesame oil (ml)	-	300	-
Mustard oil (ml)	-	-	300
Rice water (ml)	1200	1200	1200

Weighed about 300 ml of base oil and heated the till it became free from froth. Mixed 24 g of crude drug powder with fourfold quantities of rice-washed water. Boiled it on a moderate fire with constant stirring to avoid sticking the pasty mass on the bottom of the vessel. stop the fire, when the oil becomes free from water and kalka becomes mezhukupakam. The oil was filtered after cooling and packed in suitable glass jars. In this formulation, coconut oil, sesame oil, and mustard oil were base oils. The prepared oil is subjected to standardization procedures and anti-inflammatory activity studies. Analysis of oil (Vinod K Jain *et al*, 2015) done for the specific gravity, acid value, saponification value, ester value, peroxide value, iodine value, and refractive index.

In-Vitro Anti-Inflammatory Activity Studies

Pharmacological activity study conducted for both crude drug extract, golden cascade extract containing herbal ointment, all three herbal oil with different base oils like coconut oil, sesame oil, and mustard oil which containing baby coconut extract. The following studies were conducted.

i) Cyclooxygenase (COX) activity

The COX activity was assayed by the method of Walker and Gierse. 100µl cell lysate was incubated with Tris-HCl buffer (pH 8), glutathione 5 mM/L, and hemoglobin 5 mM/L for 1 minute at 25°C. The reaction was initiated by the addition of arachidonic acid 200 mM/L and terminated after 20 minutes incubation at 37°C, by the addition 200µL of 10% trichloroacetic acid in 1 N hydrochloric acid. After centrifugation and addition of 200µL of 1% Thiobarbituric acid the tubes were boiled for 20 minutes. After cooling, the tubes were centrifuged for three minutes. COX activity was determined by reading absorbance at 632 nm and percentage inhibition of COX activity was determined as per the following formula.
Percentage inhibition of the enzyme was calculated as,
% inhibition = ((Absorbance of control-Absorbance of test)/Absorbance of control) × 100

ii) Lipoxygenase (LOX) activity

The determination of LOX activity was done as per methods of Axelrod et al. Briefly, the reaction mixture (2 mL final volume) contained Tris-HCl buffer (pH 7.4), 50 µL of cell lysate, and sodium linoleate (200 µL). The LOX activity was monitored as an increase of absorbance at 234 nm (Agilent Cary 60), which reflects the formation of 5-hydroxyeicosatetraenoic acid.
Calculation
Percentage inhibition of the enzyme was calculated as,
% inhibition = ((Absorbance of control-Absorbance of test)/Absorbance of control) × 100

iii)Myeloperoxidase (MPO) activity

Cell lysate was homogenized in 50 mM potassium phosphate buffer and 0.57% hexadecyltrimethyl ammonium bromide (HTAB). The samples were centrifuged at 2000 g for 30 minutes at 4°C, and supernatant was assayed for MPO activity. MPO in the sample was activated by the addition of 50 mM phosphate buffer (pH 6) containing 1.67 mg/mL guaiacol and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was measured. MPO activity was presented as units per mL of cell lysate. One unit of MPO activity was defined as that degrading 1 µM of peroxide per minute at 25°C.

U = (ΔOD 4×Vt×dilution factor) / (L×£470×Δt×Vs)
ΔOD = Optical density change
Vt = Total volume (mL) (1.1 mL)
L = Light path (1 cm)
£470 = extinction coefficient for tetraguaiacol (26.6 mM-1·cm-1,)

iv)Estimation of Cellular Nitrite Levels

The level of nitrites was estimated by the method of Lepoivre et al. (Lepoivre et. al. 1990) To 0.5 mL of cell lysate, 0.1 mL of 3% sulphosalicylic acid was added and vortexed well for 30 minutes. The samples were then centrifuged at 5,000 rpm for 15 minutes. The protein-free supernatant was used for the estimation of nitrite levels. To 200 µL of the supernatant, 30 µL of 10% NaOH was added, followed by 300 µL of Tris-HCl buffer and mixed well. To this, 530 µL of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthyl ethylene diamine dihydrochloride) was added and incubated in the dark for 10–15 minutes, and the absorbance was read at 540 nm against a Griess reagent blank. Sodium nitrite solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves obtained.

Results and Discussion

Some references were taken from the Ayurveda for the preparation of herbal oil. The prepared oil was analysed for the physical properties and invitro anti-inflammatory activities. The results of oil analysis were shown in the **Table 2**.

Table 2: Analysis of Herbal Oil

Sl. No.	Name of base oil	Acid value	Saponification value (per g)	Ester value (wt/ml)	Peroxide value (meq/kg)	Refractive index	Specific gravity
1	Coconut oil	3.9	188	184.1	3	1.45	0.92
2	Sesame oil	4	182	178	3	1.474	0.910
3	Mustard oil	6.1	172	165.9	4	1.4640	0.918

v) Cyclooxygenase (COX) activity

As the concentration of sesame oil based herbal oil increases from 25 to 100 µg/ml, there is a gradual decrease in optical density (OD) at 632 nm, signifying reduced COX activity, alongside a corresponding increase in percentage inhibition from approximately 6% to nearly 39%. Sesame oil is already known for its anti-inflammatory activity due to the presence of sesamin, sesamolin, sesamol, and sesaminol that competitively inhibit COX enzymes, thus limiting the transformation of arachidonic acid into pro-inflammatory prostaglandins. This pharmacological inhibition leads to a significant decline in activity of enzyme, as indicated by lower OD readings, and consequently diminishes the production of inflammatory mediators (Wu et al; 2015).

Herbs in mustard oil shows a concentration-dependent decrease in LPS-induced activity, as assessed by optical density at 632nm. The percentage of inhibition rises from 8.65% at 25 µg/ml to 46.6% at 100 µg/ml. IC 50 Value – herbal oil with coconut oil base was noted to be 141.165 µg/ml, sesame oil based one was a 122.025

µg/ml, and mustard oil-based herbal oil was 101.148 µg/ml. all the values were estimated using ED $_{50}$ PLUS V1.0 Software**.** These IC50 values are connected as relative indicators of inhibitory strength—the smaller the IC50, the more effective the inhibitor. The correlation between these values indicates a hierarchy in inhibitor efficacy: an IC50 of 101 demonstrates greater inhibition compared to 122 or 141 when assessed under identical conditions. Figure 1 showed the concentration and percentage inhibition status. The series 1 represented coconut oil base, series 2 standing for sesame oil and series 3 for mustard oil base.

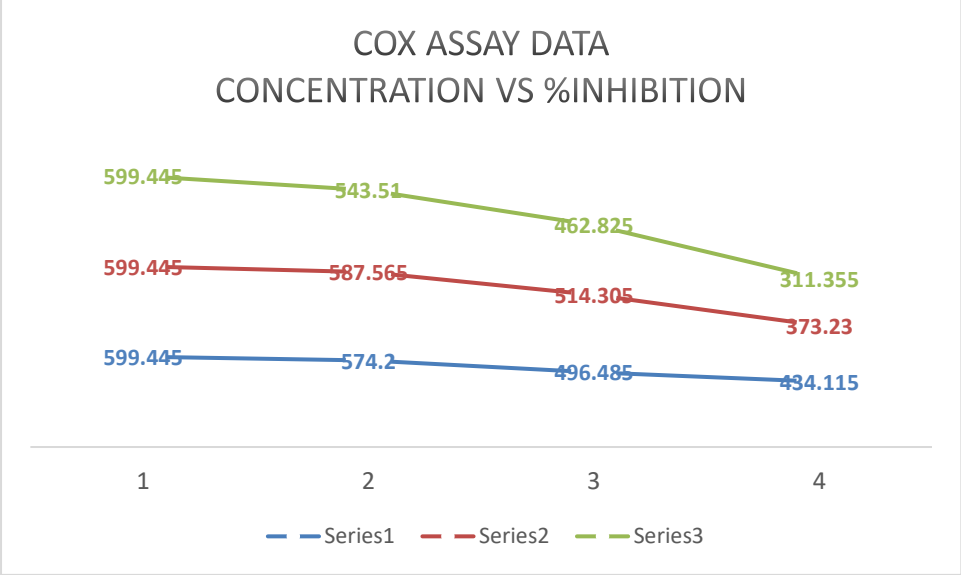

Figure 1: COX Assay

i) Lipoxygenase (LOX) activity

At 0 LPS, the measurement is 0.1251, indicating 0% inhibition.

With a concentration of 25, the measurement drops to 0.1135, reflecting approximately 9.27% inhibition. At a concentration of 50, the value further declines to 0.0935, showing around 25.26% inhibition. With a concentration of 100, the value is 0.0625, corresponding to about 50.04% inhibition. This indicates that as the concentration of LPS increases, there is a consistent reduction or inhibition in the measured parameter (potentially an activity of enzyme or an absorbance indicative of an inflammation marker or biochemical endpoint), demonstrating a dose-dependent inhibitory effect.

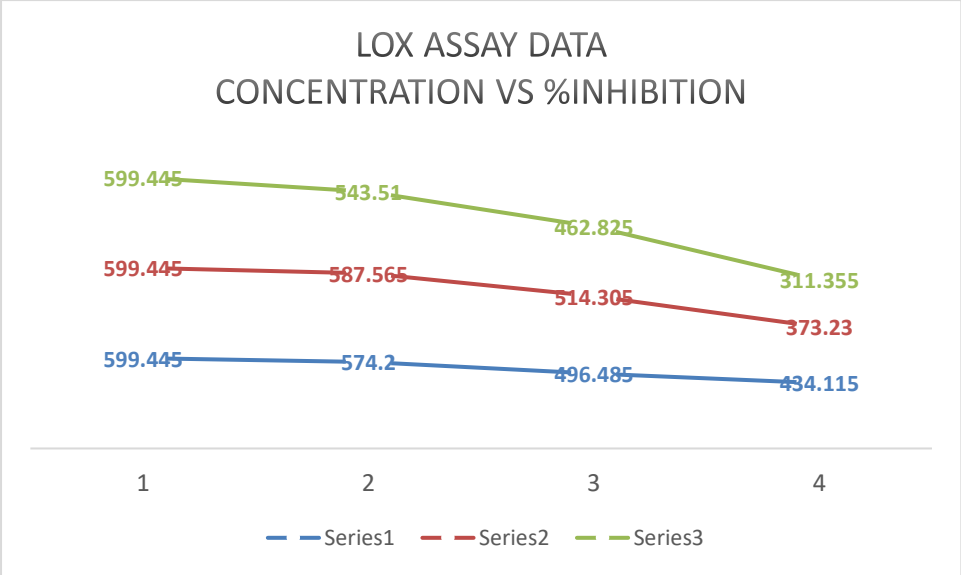

Figure 2: LOX Assay

At a concentration of 0 µg/ml (LPS only), the OD is 0.1251, resulting in 0% inhibition. At 25 µg/ml, the OD is 0.1121, indicating an inhibition of 10.39%. At 50 µg/ml, the OD measures 0.0918, corresponding to an inhibition of 26.62%. At 100 µg/ml, the OD drops to 0.0589, reflecting a 52.92% inhibition. Percentage inhibition indicates how effective a treatment is in decreasing a specific biological activity or effect, typically assessed by contrasting the sample under treatment with a control (in this case, LPS alone) and expressing the reduction as a percentage. It illustrates the extent to which the treatment—here, sesame oil based herbal oil can impede a process related to the effects induced by LPS, such as enzyme function or oxidative stress, determined by alterations in OD. The result summarised as the medicated oil exhibited a dose-dependent inhibitory effect on the LPS-induced system, as indicated by optical density measured at 234 nm. The percentage of inhibition rises from approximately 10% at a concentration of 25 µg/ml to almost 53% at 100 µg/ml, highlighting its potential anti-inflammatory or enzyme inhibitory properties quantitatively. This understanding aligns with the application of inhibition percentage in biochemical assays to evaluate the effectiveness of treatments. This indicated that increasing amounts of mustard-oil are associated with a reduction in optical density at 234 nm, signifying enhanced inhibition, which surpasses 50% at the 100 µg/ml concentration. The percentage inhibition likely indicates how effectively mustard-oil interferes with LPS-induced biological effects (such as enzymatic activity or inflammation) as measured by OD at 234 nm.

Percentage inhibition is a commonly used metric in biochemical research to evaluate the ability of substances to diminish biological functions like activity of enzyme or inflammation by comparing them to a control. The reduction in optical density (OD) at 234 nanometr as mustard-oil-based sample concentrations rise, resulting in greater percentage inhibition, suggests that it proficiently mitigates the LPS-induced biological response in a dose-dependent fashion. The highest level of inhibition remarked (~50.84%) at 100 µg/ml points to a significant reduction of LPS-induced activity (Taranu et al, 2024).

IC_{50} Values of different samples were seen to be coconut oil based herbal oil-98.9681µg/ml, when base was sesame oil-94.0171µg/ml, and mustard-oil as base -97.117µg/ml. All were calculated using ED 50 PLUS V1.0 Software. According to the IC_{50} values sesame oil base had the effective anti-inflammatory activity compared to the other two samples.

ii) Myeloperoxidase activity

Activity of enzyme (U/ml) quantifies MPO's catalytic performance, with the standard unit defined as the quantity of enzyme that breaks down one micromole of hydrogen peroxide per minute under the specified assay conditions. The details show a decline in both ΔOD and MPO activity (U/ml) as LPS concentration increases from 25 to 100 µg/mL. This decline indicates an inhibitory influence on MPO activity when higher concentrations of LPS are present, even in the presence of copra oil, which may exert modulatory or anti-inflammatory effects.

LPS is known to trigger inflammation and oxidative stress, yet elevated levels may also lead to enzyme degradation, inhibition, or depletion of substrates, which could explain this reduction.

Therefore, the performed trend can be construed as a dose-dependent inhibition or reduction of MPO activity of enzyme, indicated by changes in optical density and enzyme units, signifying a diminished inflammatory oxidative response.

In conclusion, the findings given in the **figure 3** regarding ΔOD and activity of enzyme denote the measurement of myeloperoxidase activity, which decreases as the concentration of LPS increases, implying that higher LPS concentrations suppress MPO activity within this assay framework, notwithstanding the presence of coconut oil base. This trend aligns with the principles of MPO activity assays, where enzyme performance is quantified through substrate oxidation tracked as alterations in optical density. This analysis aids in comprehending the effects of inflammatory modulation and the kinetics of MPO under conditions of LPS stimulation.

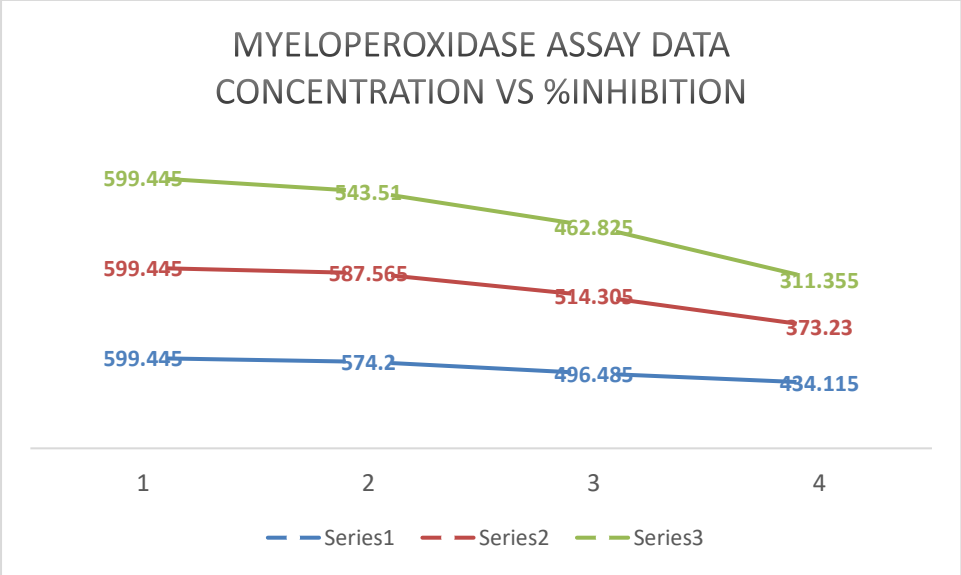

Figure 3: Myeloperoxidase activity

The information provided in **figure 3** illustrated how varying concentrations of herbal oil with sesame oil base (25, 50, 100 µg/ml) influence activity of enzyme in the presence of LPS. As the concentration of oil increases, both ΔOD and activity of enzyme (U/ml) decline, indicating that the presence of LPS resulted in inhibited activity of enzyme.

As per the records the following findings were concluded: At 25 µg/ml of sesame oil, the ΔOD measures 0.1011, and the activity of enzyme is recorded at 0.033363 U/ml. When the concentration is increased to 50 µg/ml, the ΔOD decreases to 0.0893, and the activity of enzyme falls to 0.029469 U/ml. At the highest concentration of 100 µg/ml, the ΔOD is 0.0545, and activity of enzyme drops to 0.017985 U/ml. The control measurements with LPS show a ΔOD of 0.0392 and activity of enzyme of 0.012936 U/ml.

According to existing literature, sesame oil has demonstrated anti-inflammatory effects, helping to reduce oxidative stress and lipid peroxidation caused by LPS in various experimental models. It has the ability to moderate enzyme activities associated with inflammation, particularly those linked to lipid peroxidation. The observed reduction in activity of enzyme with higher concentrations of herbal oil supports these findings, indicated that sesame seed oil base may inhibit activity of enzyme prompted by inflammation induced by LPS (Shi et al, 2016).

The information outlined the levels of mustard-oil (0, 25, 50, 100 µg/ml), the corresponding ΔOD values, and the activity of enzyme (U/ml), which appears to relate to an assay evaluating the impact of baby coconut powder with mustard-oil on enzyme activity in the presence of LPS.

From the information gathered: At 0 µg/ml mustard-oil (LPS control), the ΔOD is 0.1011 and the enzyme activity measures 0.033363 U/ml. With 25 µg/ml mustard-oil, the ΔOD is 0.0928 and the enzyme activity is 0.030624 U/ml. At a concentration of 50 µg/ml mustard-oil, the ΔOD decreases to 0.0551 and the enzyme activity falls to 0.018183 U/ml. At 100 µg/ml mustard-oil, the ΔOD further drops to 0.0419 and the enzyme activity reduces to 0.013827 U/ml.

The observed reduction in enzyme activity with higher concentrations of medicated oil in the LPS context suggests that mustard-oil may inhibit the enzyme activity assessed in this experiment (likely associated with inflammatory responses).

Regarding the enzyme activity of mustard-oil contains fatty acids such as erucic acid and linoleic/linolenic acids, which have been examined for their effects on various enzymatic hydrolysis processes utilizing lipases. Porcine pancreas lipase, exhibiting activity levels of 100-400 units/milliG of solid (where one unit is defined as the production of 1 µmole of fatty acid per hour), is frequently employed to hydrolyze mustard oil under

particular conditions, including a pH of 9, temperature of 35 °C, and with agitation. Threby the oil gave added advantage on reduction of inflammation (De and Basu, 2012).

IC 50 values of the herbal oil with different bases were 84 microg/ml, 63.25 microg/ml, and 67.2 micrg/ml for the coconut oil, sesameoil, and mustard oil accordingly. As per thedata sesamee oil base bared more efficient antiinflammation.

Estimation of Cellular Nitrite Levels

The information given in **figure 4** illustrates the impact of varying concentrations of coconut oil (µg/ml) on the nitrite levels (µg) as assessed through optical density (OD) after stimulation with LPS: At a concentration of 25 µg/ml coconut oil, the OD measures 0.1211, and the nitrite concentration is 599.445 µg. With 50 µg/ml, the OD is 0.116, and the nitrite concentration is 574.2 µg. At 100 µg/ml, the OD reads 0.1003, and the nitrite concentration is 496.485 µg. At the same higher concentration (100 µg/ml), the OD falls to 0.0877, while the nitrite concentration is 434.115 µg.

These findings indicate that higher concentrations of the base oil, coconut oil, correspond to lower nitrite levels, suggesting potential inhibition of LPS-induced nitrite production. Nitrite concentrations serve as an indicator of nitric oxide generation, which plays a role in inflammatory responses (Ng et al; 2021). The data demonstrated that herbal oil decreases both nitrite and production of NO in LPS stimulated samples in a manner dependent on dosage, thereby reinforcing its potential anti-inflammatory properties through the modulation of nitric oxide synthase activity and nitrite levels. This inhibition could mitigate the inflammatory signaling initiated by LPS in immune cells.

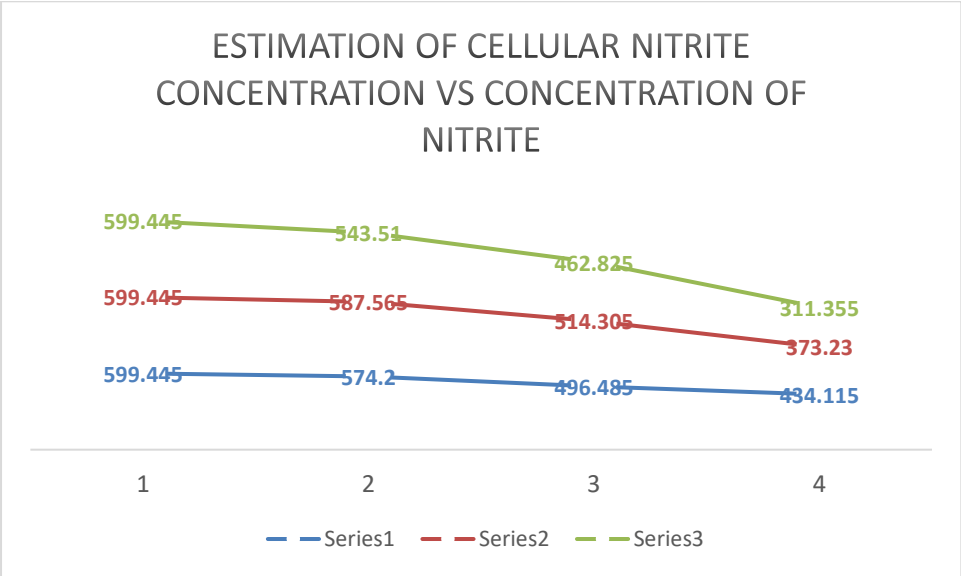

Figure 4: Estimation of cellular nitrite

As mustard oil concentration rises from 25 to 100 µg/mL, the optical density (OD) decreases from 0.1211 to 0.0629.

Similarly, the nitrite concentration declines from 599.445 µg to 311.355 µg.

This inverse correlation between the concentration of mustard oil and both OD and nitrite levels indicates that mustard oil may inhibit the production of nitrite in LPS-stimulated samples, which likely points to an anti-inflammatory effect by lowering nitric oxide production.

Here, nitrite concentration likely serves as a proxy for nitric oxide (NO) production, commonly evaluated to gauge inflammatory responses, as NO is a mediator produced by inducible nitric oxide synthase (iNOS) following LPS stimulation. The diminishing nitrite levels and OD values associated with mustardoil suggest its potential to hinder NO production, aligning with existing research on essential oils and natural substances that suppress NO synthesis and inflammatory pathways.

In conclusion, the mustardoil samples demonstrate a concentration-dependent suppression of both nitrite production and OD measurements in LPS-induced samples, underscoring its likely anti-inflammatory properties through the reduction of nitric oxide production.

The nitrite levels recorded following LPS stimulation and treatment with varying concentrations of the coconut oil-based sample exhibit a distinct dose-dependent decrease, which suggests the oil's anti-inflammatory properties. The graph **(Fig:4)** interpreted The LPS control group presents the highest nitrite level at 599.445 µg, thereby confirming a significant induction of inflammation. Upon treatment with 25 µg/mL of copra oil, nitrite levels show a slight reduction to 574.2 µg, indicating a mild inhibitory effect.

At a concentration of 50 µg/mL, the nitrite level further declines to 496.485 µg, reflecting a moderate decrease in nitric oxide production. The most substantial reduction is noted at 100 µg/mL, where the nitrite concentration decreases to 434.115 µg, signifying the most pronounced anti-inflammatory response.

Comparison of Different Base Oils

Sesáme oil offers the most balanced and robust anti-inflammatory effects, followed by mustardoil, whereas copra oil shows the least activity among the three. Sesame oil is recognized as the most effective base oil due to its strong, consistent, and multi-target anti-inflammatory activity performed across all assays. It exhibits the lowest IC_{50} values for critical inflammatory enzymes such as LOX (94.02 µg/mL) and MPO (63.25 µg/mL), indicating that it necessitates the least concentration to significantly inhibit these pathways. This illustrates a higher potency in comparison to coconut and mustardoils. The results were shown in **Table 3.**

Additionally, sesame oil effectively lowers nitrite levels, which signifies its capability to suppress nitric oxide production—an essential inflammatory mediator. Moreover, sesame seed extracted oil demonstrates a clear dose-dependent inhibition across all tests, indicating that its anti-inflammatory effects intensify progressively with increasing concentration. Collectively, these findings indicate that sesameoil offers the most balanced, potent, and dependable anti-inflammatory activity among the oils evaluated

Table 3: Comparison of anti-inflammatory effectiveness of different base oils

Parameter	Coconut oil as the base oil (µg/mL)	Sesame oil as the base oil (µg/mL)	Mustard oil as the base oil (µg/mL)	Effective oil
COX Inhibition (IC_{50})	141.17	122.02	101.15	Mustard oil
LOX Inhibition (IC_{50})	98.97	94.02	97.12	Sesame oil
MPO Inhibition (IC_{50})	84.00	63.25	67.20	Sesame oil
Nitrite Reduction at 100 µg/mL	434.11	373.23	311.35	Mustard oil
Dose-dependent Inhibition	Moderate	Strong and consistent	Strong	Sesame oil
Overall Anti-inflammatory Strength	Good	Excellent	Very Good	Sesame oil

Stability studies

Reviews disclosed that base oil like coconut oil contains a high level of saturated fatty acids (approximately 90%), which provides resistance to oxidation and hydrolysis, resulting in only slight increases in peroxide and acid values during storage at temperatures ranging from 15 to 35°COn the other hand, sesame seed oil shows moderate stability due to its natural antioxidants, sesamol and sesamin, which retard oxidative degradation.

However, its higher content of monounsaturated fatty acids renders it less stable compared to copra oil. Mustard oil, on the other hand, demonstrates the least stability due to its high quantities of PUFA (including erucic acid) and initial free fatty acids, leading to more rapid degradation even at ambient temperatures. Here the formula also revealed that the copra oil formula disclosed better stability compared to the other two. Results presented in the **Table.4.**

Table 4: Stability study of herbal Oil

Sl No.	Name of base oil	Organoleptic characters	Acid value (Initial)	After 30 days	Saponification value (per g) Initial	After 30 days
1	Coconut oil	Golden yellow color	3.9	4	188	188.2
2	Sesame oil		4	4	182	182.5
3	Mustard oil	Viscous brown	6.1	6.3	172	172.9

Summary And Conclusion

This research explored the anti-inflammatory capabilities of baby coconut integrated into herbal oils utilizing coconut, sesame, and mustard oils as base materials. Baby coconut, a multifunctional drupe from the Arecaceae family, is abundant in bioactive substances with recognized medicinal benefits, which include antioxidant, antimicrobial, and anti-inflammatory effects found throughout its husk, water, oil, and endocarp. Herbal oils were created through a conventional repeated infusion process (11 cycles over 12-15 days at 75-80°C), resulting in powerful formulations (F1: coconut oil base; F2: sesame oil base; F3: mustard oil base) that were standardized for physicochemical attributes such as acid value (3.9-6.1), saponification value (172-188 mg KOH/g), peroxide value (31-40 meq/kg), refractive index (1.45-1.47), and specific gravity (0.910-0.92).

The in vitro anti-inflammatory effectiveness was assessed in LPS-stimulated RAW 264.7 macrophages through four primary assays: cyclooxygenase (COX), lipoxygenase (LOX), myeloperoxidase (MPO), and cellular nitrite levels. Each formulation displayed dose-dependent inhibition (25-100 µg/mL), with the sesame oil-based herbal oil (F2) showing the highest overall effectiveness. IC_{50} values illustrated this ranking: COX (F1: 141.17 µg/mL; F2: 122.02 µg/mL; F3: 101.15 µg/mL), LOX (F1: 98.98 µg/mL; F2: 94.02 µg/mL; F3: 97.12 µg/mL), and MPO (F1: 84 µg/mL; F2: 63.25 µg/mL; F3: 67.20 µg/mL). Nitrite reduction at 100 µg/mL was most significant with mustard oil (311.35 µg), followed by sesame (373.23 µg) and coconut (434.11 µg), indicating a decrease in iNOS-mediated NO production.

Comparative evaluations confirmed sesame oil as the best base due to its lowest IC_{50}s for LOX and MPO, consistent dose-dependent responses, and balanced multi-target inhibition, which can be attributed to sesamin, sesamolin, and sesamol. Mustard oil excelled in COX inhibition and nitrite reduction (thanks to erucic and linolenic acids), while coconut oil displayed moderate effectiveness related to its saturated fatty acids and antioxidants. Stability assessments conducted over 30 days demonstrated that coconut oil had superior oxidative stability (minimal increase in acid/peroxide values), followed by sesame (shielded by natural antioxidants), with mustard oil being the least stable due to high PUFAs.

In summary, baby coconut herbal oil shows significant anti-inflammatory potential, with sesame oil emerging as the most effective base for strong, broad-spectrum inhibition of COX, LOX, MPO, and nitrite pathways—

making it suitable for topical products aimed at skin conditions like psoriasis, eczema, and dermatitis. Coconut oil provides the best stability for extended storage, suggesting hybrid formulations for practical use. These results confirm traditional practices and encourage further in vivo/phytochemical investigations to identify active ingredients and refine therapeutic delivery. This herbal oil presents possibilities as a natural, preservative-free substitute for synthetic anti-inflammatories in pharmacognosy-focused cosmeceuticals.

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