

Characterization and antibacterial properties of oak tasar and mulberry silkworm pupae oils

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ABSTRACT: Current study provides a valuable insight into physical, thermal, and antibacterial studies of oak tasar pupae oil (OTPO) and mulberry silkworm pupae oil (MSPO). It was observed that both OTPO and MSPO showed comparable melting ranges which were -105°C to -70°C for OTPO and -96°C to -70°C for MSPO. However, this also shows that OTPO solidifies at lower temperatures than MSPO. The viscosity of OTPO was observed as 26.16 cP, whereas, for MSPO it was 25.60 cP. The colour of the OTPO appeared as brown and yellow for MSPO which corresponds to the values 18Y + 2.1 R and 14Y + 1.0 R displayed through the tintometer, respectively. The specific gravity values OTPO and MSPO were 0.863 ± 0.0016 and 0.910 ± 0.001 respectively. The antibacterial activity of the oil samples was evaluated against *Streptococcus pyogenes* (MTCC733) and *Salmonella typhi* (MTCC442). Both OTPO and MSPO emphasized significant bactericidal abilities against the selected bacterial strains. OTPO exhibited higher antibacterial activity on gram-negative *S. typhi* of 15.33 ± 1.15 mm at 70 µL. Whereas, MSPO exhibited a maximum zone of inhibition of 13 ± 1 mm at 70 µL on gram-positive *S. pyogenes*. Further, at 20 µL, MSPO showed an inhibition of 9 ± 0 mm against *S. pyogenes*, while OTPO showed no activity against any of the strains. Furthermore, at 50 µL, the zone of inhibition of 9.67 ± 0.58 mm was observed for both OTPO and MSPO against *S. pyogenes*. Although, at the same concentration 12.67 ± 1.15 mm zone of inhibition was measured for OTPO and no activity for MSPO was obtained for *S. typhi*. The results showed significant antibacterial activity, for OTPO, which exhibited a maximum inhibition zone of 15.33 ± 1.15 mm against *S. typhi*.

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KEY WORDS: *Antheraea proylei*, *Bombyx mori*, specific gravity, viscosity, *Streptococcus pyogenes*, *Salmonella typhi*, inhibition

INTRODUCTION

The physical characteristics of insect oils, such as viscosity, density and melting point, are influenced by their fatty acid composition (Chantakun *et al.*, 2024). For instance, oils with a higher proportion of Saturated fatty acids (SFAs) tend to have a higher melting point, making them solid at room temperature, while those rich in Polyunsaturated fatty acids (PUFAs) are typically liquid. Insect oils

also exhibit unique thermal properties, which have been studied for potential applications in the food and cosmetic industries (Kim *et al.*, 2022). Beyond their nutritional and physical properties, insect oils have been found to possess significant antibacterial activity. The antimicrobial properties of insect-derived oils are attributed to the presence of bioactive compounds such as fatty acids, terpenes, and phenolics. These compounds can disrupt bacterial cell membranes, inhibit enzyme activity,

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or interfere with bacterial communication systems, thereby exerting antibacterial effects (Yan *et al.*, 2021). For example, research on the antibacterial properties of black soldier fly larvae oil demonstrated its effectiveness against Gram-positive bacteria, suggesting its potential use in food preservation and medicinal applications (Sprangers *et al.*, 2017).

Traditionally, silkworm pupae are considered a massive co-product of the sericulture textile industry. The depletion of resources with the rising global population is an alarming issue. Amid increasing food demands silkworm pupae emerged as a valuable alternative dietary supplement with high nutrition and therapeutic value (Fig. 1). In the previous study (Suman and Kumar, 2024), the authors extracted a fine amount of the pupae oils through the soxhlet method. Further, also reported the presence of conjugated linoleic acid (Ω -6 fatty acids) and other bioactive components in both oak tasar pupae oil (OTPO) and mulberry silkworm pupae oil (MSPO) through Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared (FTIR) analysis. Therefore, in the current study, aim to explore the potential physical, thermal and anti-bacterial benefits of OTPO and MSPO..

MATERIALS AND METHODS

The Indian oak tasar pupae enclosed were procured from the local seri-farmers allied to Central Tasar Research and Training Institute, RSRS Bhimtal, Uttarakhand, India. Further, pure Mysore variety of mulberry silkworm pupae encased in cocoon were purchased from the local cocoon market with the help of Central Silk Board, Bengaluru, Karnataka, India. Both the samples were freshly procured and subjected to the experiment. The pupae of both samples were taken out from the cocoons by cutting the cocoons. The pupae were air dried at 70°C in a hot air oven until the moisture was eliminated. After drying, the dried pupae were ground to a fine powder using an electric blender. The blended pupae powder (100g) was subjected for oil extraction using Soxhlet apparatus as suggested by Thirupathaiah *et al.* (2016) and

Suman and Kumar (2024). The recovered oils were kept in separate jars and labelled as OTPO for oak tasar pupae oil and MSPO for mulberry silkworm pupae oil. Both oil samples were kept in a refrigerator for further analysis.

Melting point determination by DSC: The melting point of OTPO and MSPO was determined by differential scanning calorimetry (DSC) (PerkinElmer DSC 4000). For DSC analysis, pure indium was used to calibrate the equipment, and an empty open aluminium pan was used to obtain the baseline with the sample. 3.5 ± 0.5 mg of OTPO and MSPO were weighed into open aluminium pans and placed in the equipment's sample chamber. The constant temperature was set for five distinct temperatures (100, 110, 120, 130 and 140°C), and nitrogen was used as the purge gas flowed at ~ 20 mL min⁻¹. Once the experiment was completed, the data was obtained in the form of graphs for analyses (Pradauil *et al.*, 2011).

Viscosity determination: The dynamic viscosity (μ) of OTPO and MSPO was determined by a Brookfield viscometer (USA) equipped with a thermo-container and programmable temperature controller. The oil samples were kept in the beaker and the viscometer spindle was emerged into the oil samples. Further, the motor was switched on and the spindle was allowed to rotate until a steady reading was obtained (Sahasrabudhe *et al.*, 2017).

Colour determination: The colour determination of OTPO and MSPO was done using a Lovibond tintometer. In the Lovibond method color is expressed as red and yellow components, (ISI, 1984; IS 548 (Part 1)-1964).

$$\text{Colour reading} = (a Y + 5 b R)$$

Where:

a = sum of the various yellow slides (Y) used

b = sum-total of the various red (R) slides used

Y + 5R = mode of expressing the colour of light-colored oils

Specific gravity: Specific gravity was determined as the weight of a specific volume of the OTPO and MSPO divided by the weight of a volume equivalent to the water (reference material) at room

temperature. For the specific gravity measurement firstly the weight of an empty specific bottle was recorded using a digital weight machine then the specific gravity bottle was filled with OTPO and MSPO separately and accurate weights of both the samples were recorded. After that the distilled water (DW) was filled in a clean specific gravity bottle and weight was taken (FSSAI, 2021). The specific gravities of OTPO and MSPO were determined as -

$$\text{Specific Gravity (at given temperature)} = \frac{A(T) - B}{C - B}$$

$$\text{Specific Gravity (at given temperature)} = \frac{A(M) - B}{C - B}$$

Where,

A (T) = Weight of the SG bottle containing OTPO.

A (M) = Weight of the SG bottle containing MSPO.

B = Weight of the empty SG bottle.

C = Weight of the SG bottle containing DW.

Antibacterial analysis: The antibacterial activity of the pupae oil was evaluated using agar well diffusion method against bacterial strains namely *Streptococcus pyogenes* (MTCC733) and *Salmonella typhi* (MTCC442) procured from Microbial Type Culture Collection (MTCC) Chandigarh, India. Mueller Hinton Agar (MHA) was used as a culture medium which was prepared by adding 38g of MHA in 1L of water separately. After that medium was sterilized using autoclave at 121°C and at 15 psi for 15 minutes. After sterilization, MHA plates were prepared by pouring 30mL of culture medium into sterile glass petri plates and the plates were allowed to solidify. The antibacterial efficacy of OTPO and MSPO was tested at different serial dilutions. For the serial dilution's preparation, 20, 50 and 70µL of both the oil samples were dissolved in 1 ml of hexane. Ten mg of ciprofloxacin was served as the positive control. Different dilutions of ciprofloxacin were prepared by dissolving 200, 500 and 700µg in to one ml of Hexane. The final volume of prepared serial dilutions of OTPO and MSPO (20, 50 and 70µL) and ciprofloxacin (100, 200, 300 and 400µg) was increased to 80µl by adding Hexane. Further, using a sterile cotton swab, the bacterial inoculum

was uniformly spread on agar plate. Ten minutes after spreading, wells were punched using sterile tips and each well was filled with 20µL of samples at desired concentration. The samples were allowed to disperse into the culture medium and then the MH agar plates were sealed and incubated at 37°C for 24 hours. After 24 hours of incubation, the plates were examined for the zone of inhibition which was measured in millimeters. The experiment was performed in triplicates. Hexane was used as the negative control against the bacterial strains tested (Wiegand *et al.*, 2008).

RESULTS AND DISCUSSIONS

Melting point determination by DSC: The melting point of OTPO and MSPO was determined and presented in the form calorimetric graph (Fig. 2). It was observed that both OTPO and MSPO showed comparable melting ranges which were -105°C to -70°C for OTPO and -96°C to -70°C for MSPO. However, this also shows that OTPO solidifies at lower temperatures than MSPO. The peak temperature for OTPO was -80.81°C whereas for MSPO was -79.73°C this minor difference in the peak temperatures indicates that melting OTPO is a little earlier than MSPO. MSPO exhibits a somewhat more prominent melting transition or a greater absorption of heat during the phase change, as seen by its higher peak height (1.8521mW) compared to OTPO (1.7066mW). Indicating a higher total heat absorption during melting, OTPO's larger area (81.2411mJ) and higher enthalpy change (22.8971 J g⁻¹) raise the possibility that it contains more solid components



Fig. 1 Schematic figure showing extraction of nutritious pupae oil from oak and mulberry silkworm pupae waste after the reeling process

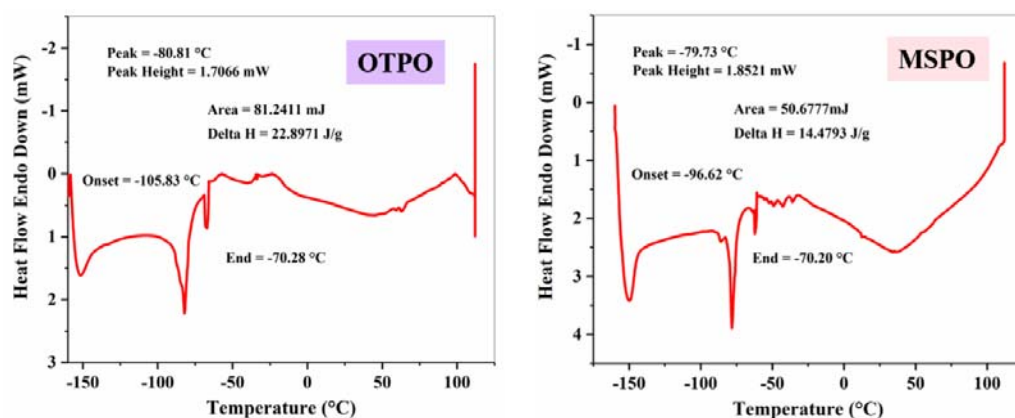


Fig. 2 Differential Scanning Calorimetric (DSC) thermograms of OTPO and MSPO showing their specific melting transitions

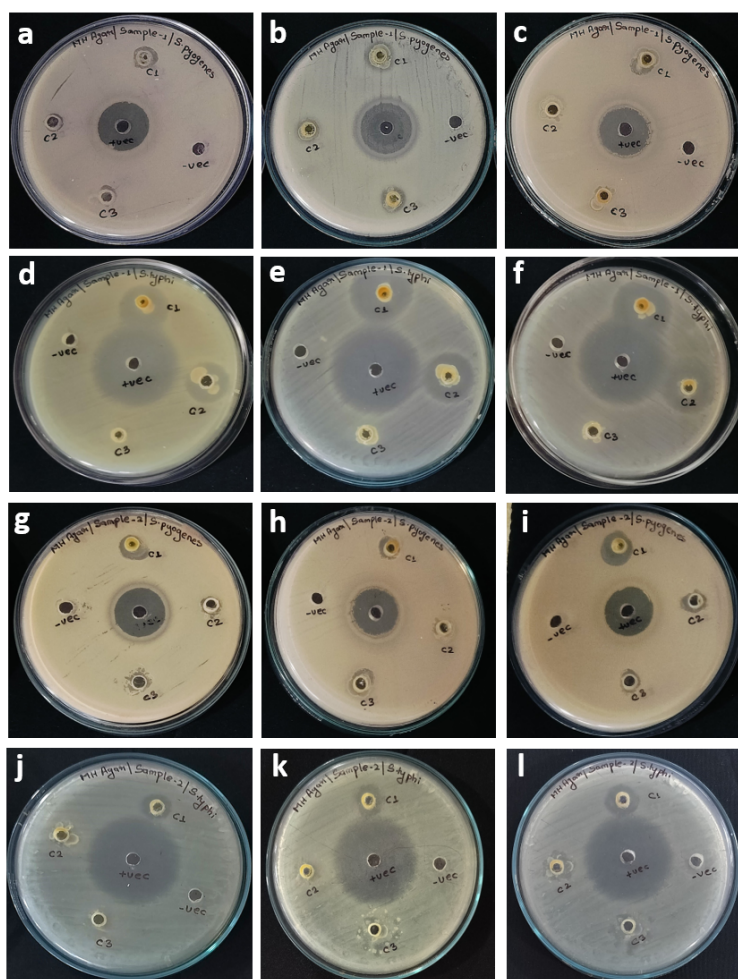


Fig. 3 Determination of antibacterial activity using Agar well diffusion assay carried on *S. pyogenes* and *S. typhi* with Oak tasar pupae oil (a–f) and Mulberry silkworm pupae oil (g–i) at different concentrations (20, 50 and 70 µL) labelled as C1, C2, and C3 respectively on plates. Standard drug Ciprofloxacin (+VEC) and hexane (-VEC) solvent used as a positive and negative control respectively against the tested bacterial strains for comparison

Table 1. Determination of physical characteristics of OTPO and MSPO

Parameters	Mean $\pm$ SD		Range for edible oils	References
	OTPO	MSPO		
Viscosity	26.16cP	25.60cP	~ 30-100cP	Diamante and Lan, 2014
Colour	Brownish yellow (18Y + 2.1 R)	Yellow (14Y + 1.0 R)	Light yellow to brown	McNicholas, 1935
Specific gravity	0.8639 $\pm$ 0.0016	0.9105 $\pm$ 0.001	~ 0.91-0.93	Ichu and Nwakanma, 2019

or a more complex mixture of fatty acids (or other components) that require more energy to melt. MSPO, in contrast, requires less energy to transform from solid to liquid than OTPO because of its smaller area (50.6777mJ) and lower enthalpy change (14.4793 J g⁻¹). Differential scanning calorimeter is a useful instrument for the determination of thermal-driven behavior including the melting point, oxidative stability, crystallization, and thermal phase transition of oils and fats especially food-grade oils such as fish oil (Tolstorebrov *et al.*, 2014), coconut oil (Srivastava *et al.*, 2017), passion fruit oil (Pardaul *et al.*, 2011), Sunflower oil (Rudakov *et al.*, 2021). Each peak in the DSC graph is associated with a detailed comprehensive physical or chemical process (Qi *et al.*, 2016). Such as the coordinates of the maxima of peaks on the abscissa axis and their areas are considerably correlated with the concentrations of basic fatty acids and triacylglycerols (Rudakov *et al.*, 2021). In the current study, DSC analysis revealed that OTPO showed a high melting point as compared with MSPO which has a low melting point. This clearly indicate that OTPO remains solid at room temperature which might be due to the presence of high concentrations of saturated fatty acids.

Viscosity determination: The viscosity of OTPO was observed as 26.16cP whereas, for MSPO it was 25.60cP (Table 1). In food processing, viscosity measurement has become a crucial and essential part of many quality control processes. It is an attribute influencing oil flow (Cullen *et al.*, 2000). The viscosity of OTPO and MSPO was reported marginally less than the eri pupae oil (Ravinder *et al.*, 2016). However, the results of the current study

were accorded with the obtained outcomes of Ferdousi *et al.* (2013) for MSPO.

Colour determination: The colour of the OTPO appeared as brown and yellow for MSPO which corresponds to the values 18Y + 2.1 R and 14Y + 1.0 R displayed through the tintometer, respectively (Table 1). The detailed measurement of the colour of fats and oils is a common and crucial criterion for the manufacturing of oils, fats, margarine, and mayonnaise (Brühl *et al.*, 2021). The Lovibond tintometer was used to determine the colour of the pupae oils. Colour is expressed as red and yellow components in the Lovibond approach. Usually, 84 Lovibond interference filters in the device are categorized into eleven groups, which include neutral, yellow, red, and blue filters, and completely refined oil maybe 0.8 (red) or 8.0 (yellow) (Zhang *et al.*, 2018). In this study, MSPO appeared yellow which is consistent with Srivastava *et al.*, (2023).

Specific gravity: Specific gravity rises when the quantity of aromatic compounds in the oil increases, whereas specific gravity falls when the amount of saturated compounds increases (Yahaya *et al.*, 2012; Abu-Ellella *et al.*, 2015). The specific gravity values OTPO and MSPO were 0.863 $\pm$ 0.0016 and 0.910 $\pm$ 0.001 respectively (Table 1). The specific gravity of OTPO was found to be similar to the value of tropical tasar pupae oil in a previous study done by Srivastava *et al.* (2024). Similarly, the value of OTPO was nearly equal to the specific gravity value of olive oil which ranges from 0.915 to 0.928 (Ichu and Nwakanma, 2019). Further, the specific gravity value of MSPO was found to be similar to the value of jojoba oil (0.863) (Gad *et al.*, 2021).

Table 2. Interpretation of antibacterial activity of OTPO and MSPO against gram-positive *S. pyogenes* and gram-negative *S. typhi*

Organisms	Zone of inhibition (mm)					
	20 μ L		50 μ L		70 μ L	
<i>S. pyogenes</i>	OTPO	MSPO	OTPO	MSPO	OTPO	MSPO
	Nil	9 $\pm$ 0	9.67 $\pm$ 0.58	9.67 $\pm$ 0.58	12.67 $\pm$ 0.58	13 $\pm$ 1
<i>S. typhi</i>	Nil	Nil	12.67 $\pm$ 1.15	Nil	15.33 $\pm$ 1.15	12.27 $\pm$ 0.46

Antibacterial activity: The antibacterial activity of OTPO and MSPO was observed through the agar well diffusion method (Fig. 3, Table 2). Both OTPO and MSPO emphasized significant bactericidal abilities against the selected bacterial strains. OTPO exhibited higher antibacterial activity on gram-negative *S. typhi* of 15.33 $\pm$ 1.15mm at 70 μ L. Whereas, MSPO exhibited a maximum zone of inhibition of 13 $\pm$ 1mm at 70 μ L on gram-positive *S. pyogenes*. Further, at 20 μ L, MSPO showed an inhibition of 9 $\pm$ 0mm against *S. pyogenes*, while OTPO showed no activity against any of the strains. Furthermore, at 50 μ L, the zone of inhibition of 9.67 $\pm$ 0.58mm was observed for both OTPO and MSPO against *S. pyogenes*. Although, at the same concentration 12.67 $\pm$ 1.15mm zone of inhibition was measured for OTPO and no activity for MSPO was obtained for *S. typhi*. Further, no intrinsic toxicity was observed by Hexane. Ciprofloxacin was used as a control to compare the obtained results.

Earlier, Suman and Kumar (2024) demonstrated a potential antibacterial activity of OTPO against *Escherichia coli*, *Enterobacter cloacae*, and *S. typhi* at different dilutions while MSPO was found to be ineffective against the tested bacterial strains using LB media. However, in the current study authors used MHA nutrient media and both OTPO and MSPO emphasized significant bactericidal abilities against the selected bacterial strains. Therefore, use of different nutrient media may also play a vital role in the evaluation. Dev *et al.*, 2017 found the antibacterial properties of *B. mori* pupae oil against *S. sciuri*. Additionally, Saviane *et al.* (2021) obtained equivalent outcomes when deploying *B. mori* pupae oil against Gram-positive

Bacillus subtilis and *S. aureus*. Likewise, Tasar pupae oil (TPO) also exhibited an antibacterial ability against *S. aureus* and *E. coli*. (Ramappa *et al.*, 2023 and Srivastava *et al.*, 2023). The results of the study reveal an effective antibacterial potential of OTPO and MSPO oils attributing to the inclusion of several bioactive components, especially MUFA and PUFA.

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