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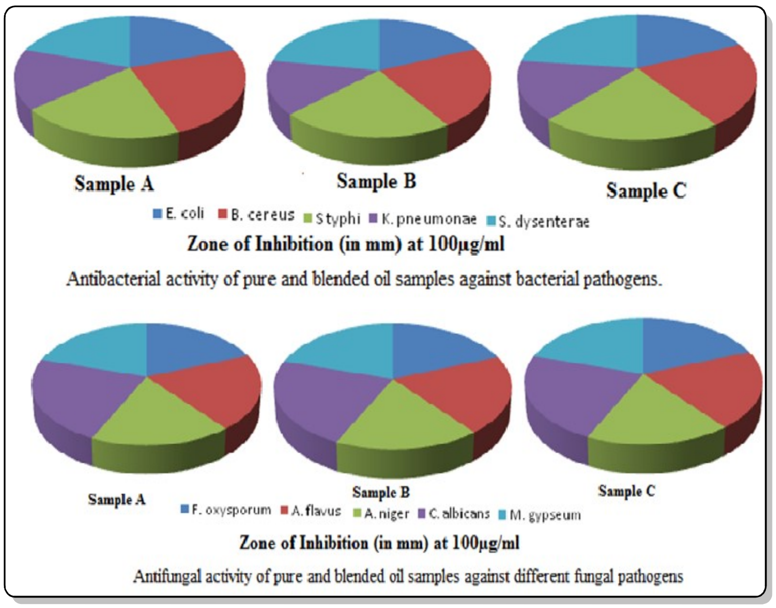
COMPARATIVE STUDY ON THE ANTIMICROBIAL ACTIVITIES OF NEEM OIL, AND MUSTARD OIL AGAINST SOME PATHOGENIC BACTERIA

Alisha Bano[1], Ajmal Tariq Hussain[2], Shahla Parveen[3], Mohammad Asif[4], Soumya Verma[5], Rizwan Ul Hasan[6]*

1Department of Pharmacy, Integral University, Lucknow, 226026, Uttar Pradesh, India.
2Amity Institute of Pharmacy, Amity University, Uttar Pradesh Lucknow Campus, 226010, India.
3Institute of Pharmaceutical Sciences, University of Lucknow, Uttar Pradesh, India.
4,5,6*Era College of Pharmacy, Era University, Lucknow, 226003, Uttar Pradesh, India.

Graphical Abstract



Abstract

The purpose of the study was to ascertain the antibacterial activity of commercially available mustard and neem oils against a variety of pathogenic microorganisms that cause disease. Using the agar disc diffusion method, the antibacterial and antifungal activity against certain organisms was investigated. When tested against specific pathogens, Both oil and mixture showed antibacterial and antifungal action. At the same concentration, neem oil had the highest rate of antibacterial activity when compared to mustard oil. According to the data, these oils can be a good source of antibacterial agents. When both oils were used in the same amount, they demonstrated synergistic antibacterial efficacy. The positive outcomes also suggest that these oils should be employed as natural antibiotics to treat various infectious disorders brought on by these pathogens. They may also help shed light on the relationships between conventional treatments and modern medications.

Keywords: Mustard Oil, Neem Oil, Antimicrobial Agents, Blending, Pathogens.

Introduction

The traditional oil-based healing treatments of the past can be regarded as highly beneficial in our day and age, when antibiotics are becoming less effective and multi-drug resistant organisms are on the rise. It is positive that people's attitudes toward traditional medicine have also improved. For many ages, several indigenous medical systems and folk remedies have employed medicinal plants to treat illnesses. Furthermore, because they are considered safer than contemporary allopathic treatments, medicinal plants are also utilized in producing herbal medicines (Ahmad *et al.,* 2013). According to estimates from the World Health Organization, 80% of people who live in underdeveloped nations receive their main medical care only from traditional practitioners. Most conventional medicinal goods' active components come from plants, and over half of the world's population still exclusively uses plants for medical purposes (Kumar & Navaratnam, 2013). Higher and more fragrant plants have long been utilized in traditional medicine and food preservation, demonstrating inhibition against yeasts, fungi, and bacteria (Hulin *et al.,* 1998). Their secondary metabolism produces essential oils, which are responsible for most of their characteristics. Both Gram-positive and Gram-negative bacteria are among the microorganisms linked to food spoiling, dental cavities, and skin conditions that can be managed with essential oils and extracts from a variety of plant species. Numerous nations have kept up research initiatives to check the antibacterial activity of traditional medicines (Sartoratto *et al.,* 2003; Akinyemi, *et al.,* 2005; Baydar, *et al.,* 2004; Bezzar-Bendjazia, *et al.,* 2017; Bouamri, *et al.,* 2015).

Oils as a therapeutic agent

Although the healing properties of essential oils have long been recognized, not many people are aware of their power to eradicate bacteria, viruses, and fungi, which help treat skin issues, prevent and treat a variety of diseases, and more. Research has demonstrated that essential oils exhibit antimicrobial, antifungal, antiviral, insecticidal, and antioxidant characteristics (Burt, 2004). Certain oils have been applied to treat cancer. A few additional oils have found application in the fragrance, aromatherapy, and food preservation sectors (Sylvestre *et al.,* 2005). Biologically active substances abound in essential oils. Examining the antibacterial qualities of plant extracts, especially essential oils, has gained more attention lately. Accordingly, it is sensible to anticipate a range of plant components with both broad and specific antibacterial activity and antibiotic potential in these oils (Singh, *et al.,* 2011; Stefan, *et al.,* 2013; Stojiljkovic. 2018; Deans. 2006; Di Pasqua, *et al.,* 2007; Dineshkumar, and Krishnakumar. 2013).

Substitute for Traditional Antibiotics

Antibiotic-resistant microorganisms and hospital-acquired illnesses remain global health issues. When used in place of antibiotics, which may have serious side effects, oils can be an excellent approach to combat bacteria. In addition to being typically safer than taking an antibiotic on prescription, they will also aid in addressing the global issue of antibiotic resistance (Warnke *et al.,* 2009). Antibiotic resistance is becoming a concern, and many experts believe that one of medicine's biggest issues of the twenty-first century is the increasing inability of antibiotics to treat infections successfully due to antimicrobial resistance. Research indicates that animals are consuming an increasing amount of antibiotics annually in addition to the overuse of antibiotics (Collignon. 2015; Lister, *et al.,* 2009; Steven, *et al.,* 2015; Stuart. 2002).

General Characteristics of Neem

Locally, *Azadirachta indica* is referred to as neem. It belongs to the Meliaceae family of mahogany trees. It belongs to the genus Azadirachta and includes one or two species. It is indigenous to Pakistan, Bangladesh, India, and Thailand. In tropical and subtropical climates, it is growing nicely. Neem fruit and seed oil are separated (Akerele, 1993; Ghimeray *et al.,* 2009). According to Hossain et al. (2013), neem is the most significant medical plant to be named the "Tree of the 21st Century" by the United Nations. The neem tree is a visually pleasing evergreen with broad leaves that can reach a height of 30 meters and spread branches that are around 10 meters in diameter. Biologically active compounds are also present in the bark and leaves, although azadirachtin- which is mostly found in seed kernels—is not present in large quantities in either. An adult tree may yield about 2 kilograms of seed annually. The majority of tropical and subtropical regions of the world currently grow trees for shade, reforestation initiatives, and the synthesis of chemicals with poisonous, antifeedant, and insect-repelling qualities on plantations (Francine, *et al.,* 2015; Khan, and Wassilew. 1987; Prates, *et al.,* 2003; Venugopalan, *et al.,* 2013).

Classification of Neem

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Sapindales
Family	Meliaceae
Genus	*Azadirachta*
Species	*Azadirachta indica*

Chemical Composition of Neem Oil

Neem oil contains 29.27% of lipids, 12.10% of proteins, and 43.28% of parietal constituents. The azadirachtin content is at 2.24 g/kg. It has 96.82% of the lipids and 92.20% of the proteins. The oil contains nine fatty acids, including four major ones oleic acid (41.91±0.69%), stearic acid (18.71±0.46%), linoleic acid (19.59±0.44%), and palmitic acid (15.59±0.27%). The oil consists of two major triglycerides: SOL by a proportion of 52.93% and POL by a proportion of 36.61%. The protein composition of neem seeds reveals the presence of 17 amino acids. The major protein is glutamic acid (23.65±0.2%), aspartic acid (9.62±0.04%), glycine (8.64±0.09%), leucine (8.09±0.11%), serine (7.19±0.24%) and alanine (7.14±0.06%).

Medicinal uses of Neem

It is used to make compounded medicine for the treatment of a wide range of human illnesses because of its medicinal value. These are listed below;

1. Neem twigs were traditionally used to brush people's teeth.
2. Its juice is said to be a beneficial tonic for boosting appetite, treating fever, or eliminating intestinal worms.
3. Neem has long been used to treat diabetes and is incurable in the Indian Ayurvedic medical system.

4. Its crude bark and leaf extracts have been used therapeutically in folk medicine to treat respiratory disorders, intestinal helminthiasis, and leprosy (Prieto *et al.,* 1999).
5. Several additional findings on the pharmacological and biological effects, including antiviral, antibacterial, antifungal, anti-inflammatory, antipyretic, antiseptic, and antiparasitic uses, are available in addition to these uses (Britto & Sheeba, 2013).

General Characteristics of Mustard

Mustard is an annual herb whose seedlings emerge fast but usually grow slowly. When the temperature and moisture content are just right, plants cover the ground in four to five weeks. The tap roots may effectively utilize the soil's stored moisture when they pierce 5 feet into the ground in dry conditions. Plants can reach mature heights of 30 to 45 inches, depending on the type, variety, and environmental factors. A brief growing season is suitable for cultivating mustard, a crop of the chilly season. While brown and oriental varieties need 90 to 95 days to develop, yellow mustard varieties typically do so in 80 to 85 days. Problems can arise from soils that are prone to crusting before seedlings develop. Soils that are too wet for this crop to grow in will result in stunted growth. Soils with dry sand and sandy loam should also be avoided. As low as 40°F in the soil will cause the seed to germinate (Oplinger *et al.,* 1991; Turgis, *et al.,* 2009; Clemente, *et al.,* 2016; Nahla E. Abd El-Aziz, *et al.,* 2021; Sultana, *et al.,* 2019).

Classification of Mustard

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Dilleniidae
Order	Capparales
Family	*Brassicaceae*
Genus	*Brassica juncea*

Medicinal uses of Mustard

1. Mustard is used topically in the form of poultices to the area where internal inflammation is located, primarily in cases of pneumonia, bronchitis, and other respiratory illnesses.
2. It helps relieve neuralgia and other pains and spasms, as well as congestion of different organs by bringing blood to the surface, as in head diseases.
3. Mustard Leaves: These are mustard seeds without fixed oil that are nonetheless Pungency-producing and sticking to paper. They are used in place of poultices.
4. When used topically in its purest form, mustard oil produces nearly immediate vesication due to its potent irritant and rubefacient properties. For rheumatism in the long run, colic, chilblains, etc., it is a very helpful use.
5. A footbath with hot water poured over smashed black mustard seeds is invigorating and can help relieve headaches and colds. It's a great fomentation as well.
6. Mustard works well internally as a mild and regular aperient while also serving as a substitute.
7. After the flour has been removed from the seeds, the bland oil that is released from their hulls encourages hair development and can be applied externally to treat rheumatism.

Chemical Composition of Mustard oil

Due to their high oil content (28–32%) and moderately high protein content (28–36%), mustard seeds have a high energy level. Mustard protein has a well-balanced amino acid composition and a high concentration of important amino acids. Up until now, mustard seeds have mostly been used to make condiments, but their beneficial chemical makeup and affordable price provide them with a lot of other uses, like adding them to meals for humans or using them to feed animals. Due to its unique fatty acid composition, mustard oil is indigestible to both humans and animals. It contains roughly 20–28% oleic acid, 10–12% linoleic acid, 9.0–9.5% linolenic acid, and 30–40% erucic acid. It is possible to decrease the high erucic acid content of mustard seeds by breeding; various nations are currently cultivating genotypes with low erucic acid content. Due to its antioxidant properties, mustard oil's high tocopherol content serves as a preservative against rancidity. When bacteria, viruses, or other microorganisms enter our bodies and start to grow, it is called an infection. The disease develops when infection damages our body's cells and telltale signs and symptoms emerge.

2 . Material and Methods

Collection of mustard and neem oils

The samples of mustard and neem oils were bought from the Nakas Bazaar local market in Lucknow, India.

Blending of oil (Neem + Mustard) oils

Mixing of the oils 50% Neem oil + 50% Mustard oil in concentrations. A 200 ml beaker was filled with 50 ml of each of the oils-mustard and neem oil which were then combined for roughly 60 minutes using a mixer. Samples that had been blended were kept for later processing and antimicrobial activity testing in a sterile glass bottle.

Evaluation of Antimicrobial Activity of the Oil Samples

Antibiotics are typically used to treat bacterial infections in the current medical system. However, the organisms frequently become resistant to antibiotic medications, and the results are not always good. Extended usage of antibiotics can result in severe adverse effects as well. The potential of the pure and blended oil samples as antimicrobials (antibacterial and antifungal) was assessed.

Investigation of in Vitro Antimicrobial Activity

Microorganisms

The bacterial and fungal cultures were obtained. The cultures were maintained on the slants and stored at 4 ^{0}C. The subculturing was done after every two months. Following microbial cultures were used for *in vitro* antimicrobial study. Bacterial cultures are *Escherichia coli, Bacillus cereus, Salmonella typhi, Klebsiella pneumonae, Shigella dysenterae* and fungal cultures are *Fusarium oxysporum, Aspergillus flavus, A. niger, Candida albicans,* and *Microsporum gypseum.* Nutrient agar medium had the following composition: The nutrient-agar medium was utilized for antibacterial activity (gm/l). To grow fungal cultures, potato dextrose agar (PDA) was used as the medium for antifungal activity, and Sabraud's agar medium was used for antifungal experiments using the plate diffusion method. PDA powder that was readily accessible and had the following pH and composition was utilized.

Plate Preparation and Determination of Antimicrobial Potential

The growth medium(s) were added to petri plates that had been previously sterilized and left to harden at room temperature. The disc diffusion method was employed to ascertain the extracts' in vitro antibacterial properties. Following a 24-hour incubation period at 370C, the cultures were subcultured in the appropriate growth medium or media. The spore suspension was made from these plates with 105 spores per milliliter. Agar medium(s) were plated with 100 µl of the suspension, and 6 mm sterile empty discs (Hi media) were collected. Various concentrations of 100 µg/ml of each oil sample were then allowed to soak on the discs. These discs were positioned on agar plates in opposition to the conventional discs containing gentamycin and nystatin as well as the control (solvent). For 24 to 120 hours, plates were incubated at 27 to 37 0C, and the zone of inhibition was monitored. The zone size was recorded by subtracting the disc diameter (6 mm). Three separate tests were run for each test. Inhibition zone size (mm) after the incubation period was measured in mm
(Prabuseenivasan, *et al.,* 2006; Rakholiya, and Chanda. 2012; Silva, *et al.,* 2011; More *et al.,* 2018).

3. Results and Discussion

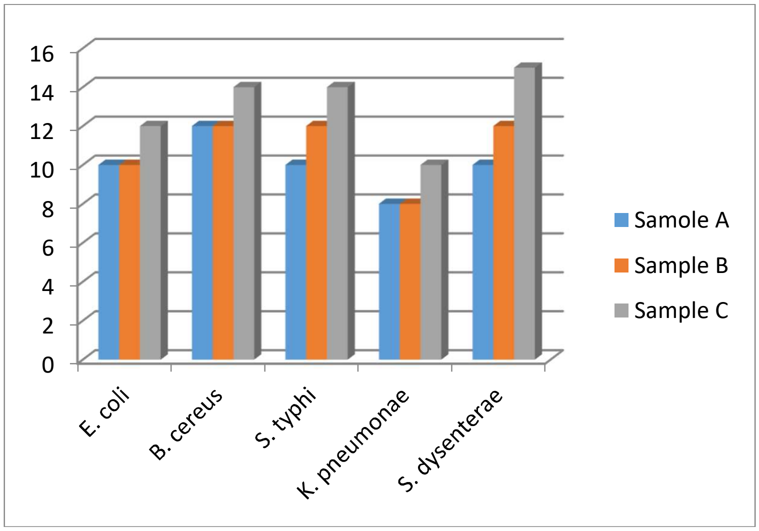
Antimicrobial activity

The antibacterial activity of several combinations produced by combining the oils of mustard and neem in varying quantities was evaluated against bacterial cultures, specifically *E. Coli, B. cereus, S. typhi, K. pneumonae,* and *S. dysenterae*. Table 1 displays the antibacterial effectiveness of several neem and mustard oil samples against the aforementioned bacterial strains. Compared to the Individual, the Combined oil sample (mustard oil and neem oil 50:50) showed significantly greater activity. *S. dysentere* had the highest level of antibacterial activity, followed by *B. cereus* and *S. typhi*. Compared to pure mustard and neem oil, it was found that the combination of oils demonstrated inhibitory effects against the majority of the microorganisms tested, ranging between 08 and 15 mm (Table 1 & figure 1).

Table 1. Antibacterial activity of pure and blended oil samples against bacterial pathogens.

S. No.	Organisms	Zone of Inhibition (in mm) at 100µg/ml		
		Oil sample A	Oil sample B	Oil sample C
1	Escherichia coli	10	10	12
2	Bacillus cereus	12	12	14
3	Salmonella typhi	10	12	14
4	Klebsiella pneumonae	8	8	10
5	Shigella dysenterae	10	12	15

A: Mustard Oil; B: Neem oil; C: Neem Oil and Mustard oil (1:1) ratio.
ZOI in mm and excludes disc diameter (6 mm).



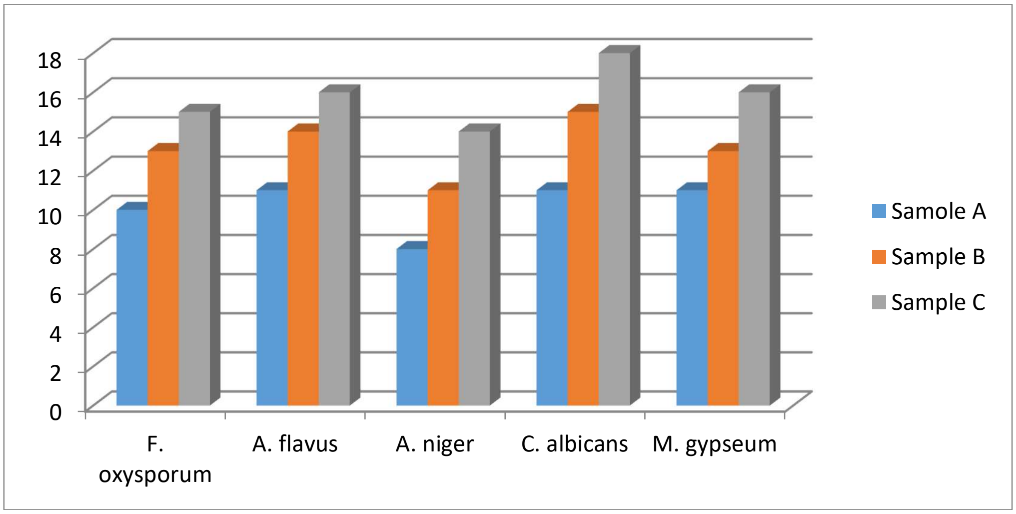
Activity against Bacterial Cultures

The antifungal activity of several combinations produced by combining the oils of mustard and neem in varying quantities was evaluated against fungal cultures, specifically *F. oxysporum, A. flavus, A. niger, C. albicans,* and *M. gypseum*. Table 2 displays the antifungal effectiveness of several mustard and neem oil samples against the aforementioned fungus strains. Compared to the Individual, the blended oil sample (mustard oil and Neem oil 50:50) showed much greater activity. The antifungal efficacy against *C. albicans* was maximal. After further examining the findings in Table 2, it was discovered that, as compared to pure mustard and neem oil, the combination of oils demonstrated inhibitory effects against the majority of the microorganisms tested, with values ranging between 08 and 18 mm (Table 2 & Figure 2).

Table 2. Antifungal activity of pure and blended oil samples against different fungal pathogens.

S. No.	**Organisms**	**Zone of Inhibition (in mm) at 100µg/ml**		
		Oil sample A	Oil sample B	Oil sample C
1	Fusarium oxysporum	10	13	15
2	Aspergillus flavus	11	14	16
3	Aspergillus niger	08	11	14
4	Candida albicans	11	15	18
5	Microsporum gypseum	11	13	16

A: Mustard Oil; B: Neem oil; C: Neem Oil and Mustard oil (1:1 ratio).
ZOI in mm and excludes disc diameter (6 mm).



Antibiotic-resistant microbes pose a threat to international health. For a variety of inexplicable causes, the prevalence of infectious diseases is rising daily. Consequently, there is a pressing need to create innovative antimicrobial medicines using readily available, naturally occurring materials like plants that don't have the harmful side effects connected with synthetic medications. Moreover, their enormous diversity makes it highly likely that they will lead to a therapeutic molecule or develop a new medicine. One of the biggest risks to the treatment of infectious diseases today is the rise of drug-resistant bacteria, which also poses a serious worldwide health and economic dilemma (Huttner *et al.,* 2013). Infections with bacteria and fungus that can be fatal are a major issue for agricultural, food, water, and medical practices. Additionally, it is unacceptable to treat infectious disorders with traditional antimicrobial drugs due to their unfavorable side effects and gradual induction of resistance (Raut, *et al.,* 2014; Helander, *et al.,* 1998; Ibrahim *et al.,* 2012). The search for new antimicrobial drugs derived from natural sources has intensified as a result. The use of plant-based herbal medicine for health treatment has been known since ancient times. Many disorders can be treated with active phytoconstituents found in plants (Padalia and Chanda, 2015). Essential oil

is one of the most promising active components of plants for the creation of new antibacterial agents. According to Pinto et al. (2013), essential oils are the result of aromatic plants' secondary metabolism, which involves a blend of many volatile chemicals. Essential oils are typically extracted from plants using steam or hydro-distillation techniques. They consist of a mixture of various low molecular-weight aliphatic hydrocarbons and terpenoids, especially monoterpenes and sesquiterpenes (Dorman and Deans, 2000). The antibacterial, antifungal, antiviral, antimycotic, and insecticidal properties of essential oils include a wide range of microorganisms, including viruses, bacteria, fungi, yeast, and more (Akthar *et al.,* 2014; Ghabraie *et al.,* 2016; Luis *et al.,* 2016). One interesting strategy to increase the efficacy of antimicrobial medicines is to mix them with essential oils (Hema, *et al.,* 2013; Hanamanthagouda, *et al.,* 2010; Frey, and Meyers. 2001; Giordani *et al.,* 2004). A complex blend of numerous phytoconstituents with distinct biological effects is called an essential oil. When chemicals are employed singly or in combination, their effects are contingent upon their concentration. Antimicrobial agent interactions can have antagonistic, synergistic, or additive effects (Bakkali *et al.,* 2008). People throughout the world have historically used certain essential oils.

4. Conclusion

It is already known that neem oil has antibacterial properties. *Brassica rapa* (mustard) and *Azadirachta indica* (neem) were the sources of oil used in this investigation. The fatty acid makeup of the mustard and neem oil samples was examined by blending them. The antibacterial activity of the oil samples, mustard oil, neem oil, and a blended neem and mustard oil mixture was demonstrated. The antibacterial properties of the blended fixed oil mixture were also assessed. The mixed neem and mustard oil showed strong antibacterial action against strains of tested bacteria and fungi, according to the results of antimicrobial activity. Oils are incredibly complicated compounds made up of several chemical components; they work very well either by themselves or in concert with other oils to efficiently combat specific bacterial strains. Antibiotic-resistant bacteria proliferate when antibiotics are overused and misused. Increased dosages have the potential to be harmful; to avoid this issue, oils with potent antibacterial properties are employed. They exhibit pharmacological synergism and exhibit bacteriocidic or bacteriostatic actions with little or nonexistent adverse effects. Oils have positive medicinal benefits, and their bioactive ingredients are beneficial. It is clear from this study that a variety of oils have antibacterial properties. The most promising bactericidal qualities are found in neem oil. The antibacterial qualities of these oils are supported by the current inquiry as well as earlier research. In poor nations, it might be utilized as an antimicrobial supplement to help create novel medicinal medicines. To support and further assess this oil's potential as an antibacterial agent in topical or oral applications, more in vivo research and clinical trials are required.

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Conflict of Interest
The authors declare no conflict of interest, financial or otherwise.

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