

In- vitro study to evaluate the antibacterial activity of *Seethodaka*, *Visarpahara* and *Neelyadi* oil against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Ayurvedic and traditional oil plays a major role in internal and external treatment for numerous diseases as well as wellness in Ayurveda. Ayurvedic studies have gifted thousands of oil recipes with dynamic applications. *Seethodaka*, *Neelyadi*, and *Visarpahara* are some of the commonly available traditional oils which show wonderful effects in treating skin diseases such as ulcers, wounds, rashes and boils. *Staphylococcus aureus* and *Escherichia coli* are the widely detected bacteria in skin conditions. This study conveys the determination of antimicrobial effects of *Seethodaka*, *Visarpahara*, and *Neelyadi* oils against *Staphylococcus aureus* and *Escherichia coli* using standard antibiotic sensitivity tests. Microbial assays were conducted using Mueller Hinton Agar medium. Five wells were made in each plate, and the bottom was sealed using molten agar. Tetracycline or Azithromycin and DMSO were used as positive and negative controls, respectively. The zone of inhibition was measured after incubation at 37°C for 24 hours. Each assay was done in triplicate and the average zone of inhibitions was calculated. The results exhibit an inhibition diameter of 12 mm for *Seethodaka* oil against *Staphylococcus aureus* while 16 mm for *Visarpahara* oil and 13 mm for *Neelyadi* oil against the same bacteria. The positive controller, Tetracycline, shows a 40mm inhibition zone diameter against *Staphylococcus aureus*. According to the results, all three oils have a considerable antimicrobial effect against *Staphylococcus aureus*. Also, 8mm of inhibition zone diameter shows for

Seethodaka oil for *Escherichia coli*, and there is an incredible antimicrobial effect shown by *Visarpahara* oil against *Escherichia coli* with an inhibition zone diameter of 24 mm. *Neelyadi* oil also has a considerable inhibition zone diameter (12mm) against *Escherichia coli*. The inhibition zone diameter of the positive controller against *Escherichia coli* is 30mm. Therefore, it can be concluded that all three tested traditional oils have considerable antibacterial effects against *Staphylococcus aureus* and *Escherichia coli*.

Keywords: *Staphylococcus aureus*, *Escherichia coli*, *Seethodaka*, *Neelyadi*, *Visarpahara*

Introduction

Ayurveda is a natural curative medical system with philosophical surroundings based on preventing and curing disease unbroken for thousands of years¹. The knowledge of Ayurveda passes from teacher to student. This huge science of medicine originated in India; however, unfortunately, due to British attacks, it became discouraged. There are eight branches of Ayurveda. *Kayachikitsa* (medicine), *Shalakyas* (dealing with diseases of the supraclavicular region), *Shalya* (dealing with extraction of foreign bodies), *Vishagaravairodika prashamana* (dealing with alleviation of poisons), *Butha vidya* (dealing with spirits), *Kaumarabhritya* (paediatrics), *Rasayana* (promotive methods), and *Vajikarana* (aphrodisiac). Five elemental theories (earth, water, fire, air, and space) and the *Thridoshaja* concept (*Vata*, *Pitta*, *Kapha*) can be considered as the basis of Ayurveda¹.

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Ayurveda has given guidelines to humans to achieve successful life with good health. It not just deals with the physical body but importantly bonding with mental health. There are four goals of Ayurveda known as *Purushartha* which need to fulfill happy life. *Dharma, Artha, Kama* and *Moksha* are them¹. Those concepts had broadly discussed in ancient Ayurvedic texts such as Charaka Samhitha, Susrutha Samhitha, and Ashtanga Hrdaya Samhitha, which are known as a greater triad (*Vrhatrai*) as well as less triad (*Laghutrai*). Curing diseases is one of the aims of Ayurveda. Different types of methods of treating various diseases have been described by different Acharyas. For those treatments, ancient Acharyas prepared several medicinal preparations processes known as *Bhaishajya kalpana*. *Bhaishajya* means a drug, and *Kalpana* means processing. With the ability of formulation, a poisonous drug can be transferred to a non-poisonous and valuable drug. Non-potentate drugs can become potent ones. Therefore, Ayurveda gave great emphasis to drug preparations under *Rasa shasthra* and *Bhaishajya kalpana*. *Bhaishajya Rathnavali* is a golden classic consisting of hundreds of Ayurvedic preparations written by Kavi Raj Govinda das². Oral medications (*Vati, Kalka, Churna, Kwatha* and *Avaleha*), ointments, oil preparations, eye drops, medicinal cigars, nasal medications, and suppositories are commonly used Ayurveda preparations for several diseases².

Among all the preparations, medicated oil is more popular in therapeutic uses³. Base oil, decoction, and *Kalka dravya* need for preparing the medicated oil. Prior to the process, crude *Thaila* should follow a special procedure known as *Thaila murchana*³. This process enhances the potency of oil and removes bad odour and *Ama dosha* of the oil³. Medicated oil is literally used externally as well as internally, for a large number of diseases. Hemiplegia, frozen shoulder, diseases of the oral cavity, diseases of the ear, dental problems, facial paralysis, greying hair, baldness, skin diseases, sciatica, ulcers, eczema, and psoriasis are a few of them. Ayurvedic and traditional oil has a major role in the number of treatments of Ayurveda. It has outstanding

pharmacological actions which reach high results in a wide range of diseases. There are thousands of oil recipes can be found in ancient Ayurvedic texts as well as traditional texts. *Aushada Samgrahaya*, *Purana Rahas Thel Beth Potha*, *Yoga Ghnadeepani*, and some ola leaves reveal valuable recipes oils in traditional Sri Lankan medicine while *Bhaishajya Rathnavali* is rich with valuable Ayurvedic recipes. Various herbal oil use in ayurveda for *Abhyanga* (massage), *Nasya* (nasal drops), *Shiro dhara*, *Sarvangadhara*, *Kavala*, *Ghandusha* and also used in the application for skin diseases. *Pinda* oil, *Seethodaka* oil, *Jaathyadi* oil, *Neelyadi* oil, and *Sarvavishadi* oil are commonly used for skin diseases in Sri Lanka.

In the present context, human beings are highly susceptible to a variety of skin diseases. It may be due to infection, allergies, autoimmune diseases, and ulcers caused by an external force and ulcers caused by some diseases such as diabetes. Shingles, urticaria, psoriasis, eczema, blisters, rashes, acne, and diabetic ulcers are some of the common skin lesions. Skin infection is common among all skin diseases, which range from mild to life-threatening. According to dermatologists, there are four types of skin infections: bacterial, viral, fungal, and parasitic. Cellulitis, impetigo, boils, and leprosy are a few of the most common bacterial infections, while shingles, chicken pox, and measles are common viral infections. Yeast infection, athlete's foot, ringworm, and oral thrush can be considered as common fungal infections. Bed bugs, scabies and cutaneous larva migrants are considered as common parasitic infections⁴.

Microbes are highly abundant in common environmental niches and perform various roles to balance the environment. In humans and animals, they provide vast protection against foreign bodies, help to build up immunity, produce some vitamins, and facilitate the digestive process. But researchers have identified that a number of microbes, and their genes badly affect humans and produce diseases⁵. Skin is the first barrier against foreign invaders and also it is the habitat for a diverse population of microbes. *Staphylococcus aureus* (*S. aureus*),

Streptococcus pyogenes (*S. pyogenes*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Candida albicans* (*C. albicans*), and *Escherichia coli* (*E. coli*) can be considered as common pathogenic microbes that grow over skin lesions⁵. *S. aureus* is a gram-positive facultative anaerobe bacterium. Sometimes it may be a skin commensal in approximately 20% of the population⁵. *S. aureus* produces multiple enzymes as well as toxins to attack the host immune system and establish infection. *Pseudomonas aeruginosa* is a gram-negative opportunistic bacterium that commonly infects patients with cystic fibrosis, burn wounds, COPD, cancer, and immunodeficiency⁶. *P. aeruginosa* infection is difficult to treat due to natural resistance of it. It shows resistance to commonly used antibiotics such as aminoglycosides, carbapenems, and cephalosporins⁶. *E. coli* is a gram-negative bacteria and an important member of the normal intestinal microflora of mammals. According to several research findings, Ayurvedic and traditional medicinal preparations are supposed to have incredible antimicrobial effects. This study is oriented towards the evaluation and comparison of the antibacterial activity of *Seethodaka* oil, *Visarpahara* oil, and *Neelyadi* oil.

Seethodaka oil is a traditional oil which commonly practices in traditional medicine for skin diseases (rashes, ulcers, itching). The main ingredients of *Seethodaka* oil are the *Kaha* (*Acorus calamus*), *Hatavariya* (*Asperagus racemosus*), and *Kohomba* (*Azadirachta indica*) with coconut oil. A paste added to the main ingredient is known as *Kalka* which is made with *Kottamalli* (*Coriandrum sativum*), *Sadikka* (*Myristica fragrans*), *Suduru* (*Cuminum cyminum*), *Kaluduru* (*Nigella sativa*), *Tippili* (*Piper longum*), and *Karabu* (*Syzygium aromaticum*)⁷. *Acorus calamus* is one of the major ingredients in *Seethodaka* oil. In Ayurveda, *Acorus* is considered a plant with an outstanding germ-killing effect. *Acorus* wear as a bracelet in Asian countries to protect from minute germs⁸. *Asperagus racemosus* is another major ingredient found in the aforementioned oil. According to Ayurveda, the common therapeutic indications of *Asperagus* are

skin diseases, skin rashes, epilepsy, urinary disorders, gynaecological problems, improper lactation, and joint disorders⁸. *Azadirachta indica*, another major compound, has common therapeutic indications for skin diseases, itching, burning sensation, anorexia, fever, and blood disorders. *Visarpahara* oil is also a herbo-mineral traditional oil that is commonly used in a number of skin diseases. *Manoshila* (Arsenic sulphide), *Gandaka* (Sulphur), *Palmanikkam* (Copper sulphate), *Sivanguru* (Fe_2O_3), *Sadikka* and *Vasavasi* (*Myristica fragrance*), *Suduru* (*Cuminum cyminum*), *Masakka* (*Hyssopus officinalis*), *Ulaarisi* (*Buceras foenum*), *Kaluduru* (*Nigella sativa*), *Tippili* (*Piper longum*), *Chopachini* (*Smilax glabra*), *Karabu* (*Zyzygium aromaticum*), *Katukarosana* (*Picrorrhiza kurroa*), *Uluhal* (*Trigonella foenum*), *Valangasal* (*Embelia ribs*), *Asamodagam* (*pimpinella anisum*), *Heen aratta* (*Alpenia calcarata*) are the ingredients of *Visarpahara* oil. Almost all the ingredients show excellent effects on skin diseases individually and are commonly used in traditional medicine. Out of all ingredients *Gandaka* (Sulphur), *Palmanikkam* (Copper sulfate), and *Cuminum cyminum* have a great curative effect on skin diseases^{9,10}. *Neelyadi* oil is a precious traditional oil that is highly applicable for a wide range of medical conditions such as skin rashes, ulcers, dermatitis, headache, and scalp diseases as well as it is commonly used in traditional *Kadumbindum wedakama* (medicines for fractures). Major ingredients of *Neelyadi* oil are, *Madatiya* leaves and bark (*Adenantha pavonina*), *Ankenda* leaves and bark (*Acronychia pedunculata*), *Vel keppetiya* leaves and bark (*Croton coudatus*), *Diyahabarala* (*Monchoria vaginalis*), Coconut milk, Coconut oil, *Sudulunu* (*Allium sativum*), *Kaluduru* (*Nigella sativa*) and *Suduru* (*Cuminum cyminum*)⁷. *Sadikka* and *Vasavasi* (*Myristica fragrance*), *Karabu* (*Syzygium aromaticum*), *Suduru* (*Cuminum cyminum*), *Asamodagam* (*Trachispermum ammi*), *Athividayan* (*Aconitum hetarophyllum*), *Tippili* (*Piper longum*), *Kaluduru* (*Nigella sativa*) and *Katukarosana* (*Picrorrhiza kurroa*) are used as *Kalka dravya* of *Neelyadi* oil.

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Materials and methods

Materials

Mueller-Hinton agar, Nutrient broth, Distilled water, Conical Flasks, Petri plates, Cork borer, Chemical balance, Micropipettes.

Methods

Anti-bacterial assay

Microbial assays were conducted using Mueller Hinton Agar medium. An amount of 37.0 g of Mueller Hinton agar and 3.0 g of bacteriological agar were weighed and dissolved in 1 L aliquot of distilled water. The mouth was tightly covered with cotton wool and autoclaved at 121°C for 15 minutes. The medium was poured into sterile culture plates inside the biosafety cabinet. *Neelayadi*, *Visarpahara*, and *Seethodaka* oils were tested for antibacterial activity against standard-type strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25922). A loopful of culture was added to a 5 mL aliquot of saline and incubated overnight at 37°C. The suspension was compared with the McFarland standard.

Five wells were made in each plate and the bottom was sealed using molten agar. Tetracycline (1000 ppm, 50µL for *Staphylococcus aureus*) or Azithromycin (1000 ppm, 50µL for *E. coli*) and DMSO (10 %, 50 µL) were prepared. An aliquot of 25.00 µL from *Neelayadi* oil, *Visarpahara*, and *Seethodaka* oil was separately placed into the wells by dissolving DMSO (10 %, 25 µL). Tetracycline or Azithromycin and DMSO were used as positive and negative controls, respectively. The zone of inhibition was measured after incubation at 37°C for 24 hours. Each assay was done in triplicate, and the average zone of inhibitions was calculated.

Results

Antibacterial Assay plate description for *S. aureus* is shown in Figure 1.

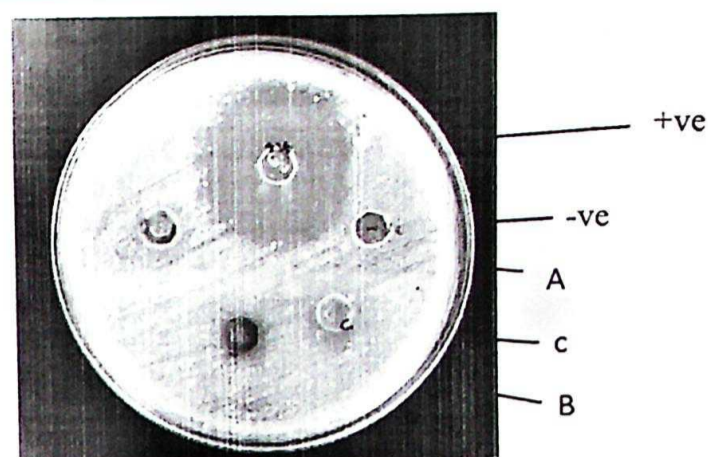


Fig. 1: Antibacterial Assay plate description for *S. aureus*. (A – *Neelyadi* oil, B- *Seethodaka* oil, C- *Visarpahara* oil)

Table 1 shows the results of the tested oils against *Staphylococcus aureus*

Oil	Test micro organism	Test drug inhibition zone diameter (mm)	Positive control inhibition zone diameter	Negative control inhibition zone diameter
<i>Seethodaka</i> oil	<i>S. aureus</i>	12mm	40mm	00mm
<i>Visarpahara</i> oil	<i>S. aureus</i>	16mm	40mm	00mm
<i>Neelyadi</i> oil	<i>S. aureus</i>	13mm	40mm	00mm

Figure 2 shows the Antibacterial Assay plate description for *E. coli*.

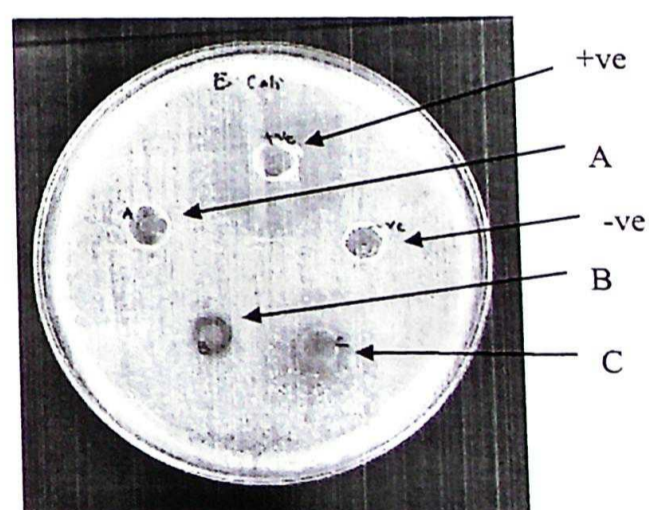


Fig. 2: Antibacterial Assay plate description for *E. coli*. (A –*Neelyadi* oil, B- *Seethodaka* oil, C- *Visarpahara* oil)

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The results of the tested oils against *E. coli* are shown in Table 2.

Table 2: Results of the tested oils against *E. coli*

Oil	Test micro organism	Test drug inhibition zone diameter (mm)	Positive control inhibition zone diameter	Negative control inhibition zone diameter
<i>Seethodaka</i> oil	<i>E. coli</i>	8mm	30mm	00mm
<i>Visarpahara</i> oil	<i>E. coli</i>	24mm	30mm	00mm
<i>Neelyadi</i> oil	<i>E. coli</i>	11mm	30mm	00mm

Discussion

The results exhibit an inhibition diameter of 12mm for *Seethodaka* oil against *S. aureus* while 16mm diameter for *Visarpahara* oil and 13mm of diameter for *Neelyadi* oil against the same bacteria. The positive control, tetracycline shows a 40mm inhibition zone diameter against *S. aureus* (Table 1). According to the results, all three oils have a considerable antimicrobial effect against staphylococcus aureus. Staphylococcus aureus is a gram-positive bacterium that is the leading cause of skin and soft tissue infections such as abscesses, furuncles, and cellulitis¹¹. Abscess is the pocket of infection and is usually filled with pus and has inflammatory signs such as redness, pain, and warmth. Cellulitis is an infection of underlying layers of the skin that mostly occur in the legs or arms¹¹. It also shows inflammatory signs. According to the results, *Seethodaka*, *Visarpahara*, and *Neelyadi* can clinically apply to skin diseases such as Abscesses, Furuncles, and cellulitis due to their antimicrobial activity against *S. aureus*.

According to the results (Table 2), 8mm of inhibition zone diameter shows for *Seethodaka* oil against *E. coli*, and an incredible antimicrobial effect is shown by *Visarpahara* oil against *E. Coli* with an inhibition zone diameter of 24 mm. *Neelyadi* oil also has a considerable inhibition zone diameter (12mm) against *E. coli*. The inhibition zone diameter of positive control against *E. coli* is 30mm. The

primary habitat of *E. coli* is the gut but it may survive on skin due to unhygienic conditions as well as skin infections¹². *E. coli* strains are isolated from skin and skin infections, confirming their presence. The results indicate all three oils have antimicrobial activity against *E. coli* and can apply to skin conditions that lead to generating harmful effects by *E. coli*.

Visarpahara oil has a potent antibacterial effect against both *E. coli* and *S. aureus* compared to the other two oils. The number of mineral ingredients such as sulphur, *Manoshila* (Arsenic sulphide), Copper sulphate, and *Sivanguru* (Fe_2O_3) of *Visarpahara* oil may lead to having an enhanced antibacterial effect than the other two oils. In Ayurveda, *Rasa shashtra* has mentioned a number of mineral compounds for several diseases and those are more effective than herbal compounds. *Gandaka* (sulphur) and its preparations are used for skin diseases for thousands of years¹⁰. Sulphur has an amazing antibacterial effect against Staphylococcus aureus¹³. It is important in the management of dermatological conditions such as scabies, acne, and dandruff¹⁴.

Conclusion

All tested traditional oils of *Seethodaka*, *Visarpahara*, and *Neelyadi* have a considerable antibacterial effect against *S. aureus* and *E. coli*. Therefore, all three oils can effectively be used for managing skin conditions caused by *S. aureus* and *E. coli*. Among the tested three oils, *Visarpahara* oil shows the highest inhibition zone diameter against both *S. aureus* and *E. coli* than *Seethodaka* oil and *Neelyadi* oil. *Visarpahara* oil shows the highest inhibition zone diameter for *E. coli* (24mm) than *S. aureus* (16mm). *Seethodaka* oil shows a high inhibition zone diameter for *S. aureus* than *E. coli*. *Neelyadi* oil also shows a better antimicrobial effect against *S. aureus* than *E. coli*.

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