

# GLOBAL ACADEMIC RESEARCH INSTITUTE

COLOMBO, SRI LANKA



## GARI International Journal of Multidisciplinary Research

ISSN 2659-2193

**Volume: 09 | Issue: 02**

On 30<sup>th</sup> June 2023

<http://www.research.lk>

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GARI Publisher | Siddha Medicine | Volume: 09 | Issue: 02

Article ID: IN/GARI/JOU/2022/107A | Pages: 33-38 (06)

ISSN 2659-2193 | Edit: GARI Editorial Team

Received: 15.05.2023 | Publish: 30.06.2023

# IN VITRO EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF SEED OF PAPAVER SOMNIFERUM AGAINST STAPHYLOCOCCUS AUREUS

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## **ABSTRACT**

Due to the developing antibiotic resistance against dangerous bacteria, the search for scientific approval of novel medications derived from plants continues. In Sri Lanka, Papaver somniferum is used to cure infectious disorders. As a result, the disk diffusion method was utilized to assess the in vitro antibacterial activity of Papaver somniferum seed extracts against Staphylococcus aureus. Long regarded as one of the most significant microorganisms that harm humans through sickness, Staphylococcus aureus. It was used to gather and extract the seeds of Papaver somniferum. Mark the location of the control, benchmark, and petri dish test areas. Prepare the culture medium and allow the Staphylococcus aureus to develop. The test was run using the conventional disc diffusion assay technique. Papaver somniferum seed crude extracts in concentrations of 100%, 50%, and 25% were dissolved to create sample discs. The diameter of the zone of bacterial growth inhibition demonstrated that the different concentrations (100%, 50%, 25%) of seed powder of Papaver somniferum aqueous extract were more effective against Staphylococcus aureus, with mean values of the hot extract being 1.960.54Cm, 1.130.38Cm, and 0.8330.77Cm, and mean values of the cold extract being 1.531.11Cm, 1.060.61Cm, and 0.80. When comparing the effects of hot and cold extracts on Staphylococcus aureus, hot extract with an IDOM of 100%

had a more pronounced effect (1.96 0.54 cm). The powdered seeds of Papaver somniferum can be employed as an antibacterial agent, according to the study's conclusion.

Key words: Papaver somniferum, Staphylococcus aureus, in vitro antibacterial activity

## **INTRODUCTION**

Medicines known as antibiotics are used to both prevent and treat bacterial infections. When bacteria adapt to the use of antibiotics, antibiotic resistance develops. The way antibiotics are prescribed and used in the world needs to alter immediately. Without a change in behavior, antibiotic resistance will continue to pose a serious concern even if new medications are created<sup>1</sup>. Infectious endocarditis, osteomyelitis, bacteremia, and deadly pneumonia are just a few of the infectious disorders that Staphylococcus aureus (S.aureus) can cause. It is also one of the leading pathogens in hospital and community infections<sup>2</sup>.

One of the main nosocomial pathogens in Sri Lanka that causes minor to serious infections is Methicillin-resistant Staphylococcus aureus (MRSA). MRSA prevalence in Sri Lanka ranges from 47% to 62% in hospital settings, and the majority of cases are resistant to the majority of tested antibiotics<sup>3</sup>. About 30% of healthy persons have S.aureus on their skin, and about 20% have it in their noses

(typically just temporarily).Mild to fatal infections caused by Staphylococcus aureus can occur. Skin infections, which frequently result in abscesses, are the most prevalent staphylococcal infections. The bacteria can, however, spread via the circulation (a condition known as bacteremia) and infect nearly any area of the body, most notably the bones (osteomyelitis) and heart valves (endocarditis). They can also cause pneumonia in the lungs. There are numerous S.aureus strains. Some strains release toxins that can result in scalded skin syndrome, toxic shock syndrome, or staphylococcal food poisoning<sup>4</sup> (Sharp, 2021).

Khash khash, also known as Papaver somniferum, is a member of the Papaveraceae family. It is one of those common plants with a long history of medicinal use. The most significant component of the opium poppy (Papaver somniferum) is the production of some of the most popularly used pharmaceutical alkaloids, including morphine, codeine, thebain, and porphyroxine. In addition to these alkaloids, the opium poppy also yields about 80 other alkaloids that fall into distinct tetra hydro benzyl isoquinolone-derived groups. Since more than a century ago, it has been understood that morphinan alkaloids build up in the opium poppy's latex. Modern literature and research studies also attest to its therapeutic usefulness, which it possesses the most significant therapeutic properties. It can be utilized as a narcotic, stimulant, sedative, analgesic, nutritional, etc. Additionally, it helps with headaches, coughing, sleeplessness, cardiac asthma, and biliary colic<sup>5</sup>. Despite the recent interest in drug discovery using molecular modeling, combinatorial chemistry, and other synthetic chemistry methods, natural product-derived compounds are still proving to be an invaluable source of medicines for humans<sup>6</sup>

Papaver somniferum has been the subject of numerous studies for its pharmacological properties, including demulcent, nutritive, and astringent. In the Siddha text volumes, a number of medications of plant, mineral, and animal origins are discussed for their antibacterial effects. However, no scientific proof was discovered for the antibacterial properties of Papaver somniferum seed. In order to research the antibacterial activity against Staphylococcus aureus, the antibacterial impact of Papaver somniferum seed was chosen.

## **LITERATURE REVIEW**

### **Papaver somniferum L**

The poppy, or Papaver somniferum L., is a plant with several uses. Its seeds and oil are used as food, while its blooms and dry capsules are used as beautiful plants. Additionally, it has medical uses. P. somniferum thrives in climates with long, hot summers and moderate rainfall. Turkey, India, Australia, France, Spain, and Hungary are the top producing nations. Turkey accounts for about 51% of the area utilized to grow poppies globally. It is regarded as a useful source for dietary supplements because of its high linoleic acid content. Additionally, because tocopherols and sterols have beneficial nutritional and anti-oxidative properties, interest in cold-pressed poppy seed oil is growing every day<sup>7</sup>.

The Kingdom is Plantae, Subkingdom is Viridiplantae, Division is Tracheophyta, Class is Magnoliopsida, Super order is Ranunculanae, Order is Ranunculales, Family is Papaveraceae, Genus is Papaver L, Species is Somniferum L, there is Bionomial name is Papaver Somniferum L, Other names are in english Opium poppy, like wise in Tamil, Kasakasa & Sanskrit is Khas-Khas<sup>8</sup>.

## **Morphology, Origin, Distribution and chemical composition**

Poppies are grown as attractive flowers and grow to a height of 3 to 4 feet. They offer a variety of exquisite blooms in the colors red, white, purple, mauve, and pink. Within two to three weeks of sprouting, poppies grow one to three primary stems that emerge from the middle of the basal rosette. Each stem has lacy foliage and one flowering bud at the end, which bends or nods until the time comes to bloom<sup>9</sup>.

Simple leaves are those that are lobed or unlobed but are not divided into leaflets. One leaf is present at each node along the stem in an alternate leaf pattern.

Edges of leaf blades, leaf blade edges with lobes, or leaf blade edges with both teeth and lobes, the leaf blade's edge has teeth<sup>10</sup>.

Flower: 8-10 cm broad, regular (actinomorphic). Four petals, often crimson but occasionally white, pale, or dark red. 2nd sepals drop as the bloom opens, numerous stamens, many joined carpels in the pistil. 1-3-flower inflorescences are with single blooms ending branches. The bud is Egg-shaped and lengthy. July to August is when flowers bloom<sup>11</sup>. (Kasviatlas, 2010).

Fruit: A glabrous, oval, 13–27 mm (0.5–1.08 in) long capsule. Stigma-disc typically has 8 to 12 rays. Poppy seed is a tiny, dried opium poppy seed that is used to flavor food and is the source of poppy seed oil. Poppy seeds are not narcotic because the fluid that would eventually turn into opium is only present in the bud before the seeds are fully formed. The kidney-shaped, tiny, grayish blues to dark blue seeds have a length of around 1 mm (0.04 inch). They are particularly well-liked in breads and other baked goods because of their mild, nutty flavor and nut-like scent. Linoleic and oleic acids are the main constituents of the fixed oil that makes up between 44 and 50 percent of poppy seed<sup>12</sup>.

The proximate analysis of poppy seeds revealed that they had the following amounts of protein, moisture, ash, crude fines, and total carbohydrates: 440 g kg<sup>-1</sup>, 211 g kg<sup>-1</sup>, 50 g kg<sup>-1</sup>, and 62 g kg<sup>-1</sup>. The main components of the poppy seeds were calcium and potassium. The predominant saturated fatty acid was palmitic acid (86-4 g kg<sup>-1</sup>), while the main unsaturated fatty acid was linoleic acid (750 g kg<sup>-1</sup> total fatty acids). In poppy seed oil, there were 220 g/g, 40 g/g, and 20 g/g of -, -, and - tocopherols, respectively. Niacin, thiamin, and pantothenic acid were discovered to be the three water-soluble vitamins with the greatest levels, respectively<sup>13</sup>.

## **MATERIAL & METHODS**

Papaver Somniferum Seeds, one of the spices utilized in the study, were purchased from a local Siddha Ayurvedic Medical store in the Trincomalee district. The Gunapaadam Division of the Unit of Siddha Medicine, Trincomalee Campus, Eastern University, Sri Lanka, received the voucher for these plants and spices, which was then taxonomically authenticated. After a thorough wash with tap water, the seeds were dried in a sun shed. The dried seeds were ground into a powder and then placed in an airtight glass container.

### **Extract preparation**

#### **Cold water extraction**

Two grams of powder were mixed with 10 milliliters of distilled water, thoroughly crushed with a motor and mortar, and shaken for 24 hours. The mixture was then centrifuged for 10 minutes at 10,000 rpm. According to Paheerathan (2021), the supernatant was carefully removed and kept at room temperature.

#### **Hot water extraction**

In 10ml of distilled water, two grams of powder were added and thoroughly

ground with a mortar and pestle. After that, it spent 5 minutes in a water bath (100). After letting it cool, it was centrifuged at 10,000 rpm for 10 minutes. Carefully separated and kept at room temperature is the supernatant. In order to determine the ideal concentration in hot and cold extracts, several concentrations of 25%, 50%, and 100% were created<sup>14</sup>.

### Preparation of müeller-hinton Agar

According to the manufacturer's recommendations, Meller-Hinton agar was made from a commercially available dehydrated base. Immediately following autoclaving, it was allowed to cool in a water bath at 45 to 50 degrees Celsius. A uniform depth of around 4 mm was achieved by pouring the prepared agar solution and cooling the medium into glass flat-bottomed petri dishes on a level, horizontal surface. For plates with a diameter of 150 mm, this equates to 60 to 70 ml of medium, while for plates with a diameter of 100 mm, it equals 25 to 30 ml. Unless the plates will be kept in a refrigerator (2 to 8C), the agar medium was left at room temperature<sup>14</sup>.

### Selection and source of micro-organism

Bacterial culture was collected from the Microbiology laboratory of the Faculty of Health Care Sciences, Eastern University of Sri Lanka, in order to evaluate the antibiotic activity of the extracts. *Staphylococcus aureus*, a gram-positive bacterium, was the bacterial strain. The culture was subcultured on Meller-Hinton agar and incubated for 24 hours at 37°C. Until their next use, the subcultures were kept at 5 °C<sup>14</sup>.

### Preparation of inoculums

Bacteria from the subcultures were dissolved in saline solution under aseptic conditions for the disk diffusion experiment. This was streaked against petri dishes containing Meller-Hinton agar

and evaluated against the 0.5 MacFarland standard to achieve a bacterial concentration between 1-2 x 10 colony forming units per milliliter (CFU/ml). This was done under sterile conditions. Filter paper disks were then added. Distilled water served as the negative control, and amoxicillin served as the positive control<sup>14</sup>.

### 3.4. Preparation of positive control

Amoxicillin was made from 500 mg pills, diluted water was used to make the final stock concentration, and filter sterilization was performed.

## RESULTS & DISCUSSION

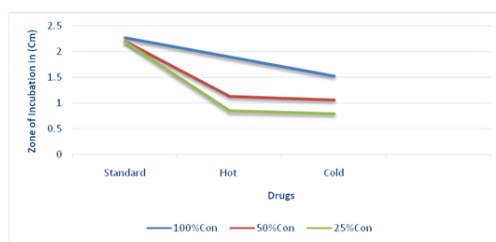


Figure 1- Effectiveness of *Papaver somniferum*'s seed against *Staphylococcus aureus* indifferent concentration of Hot & Cold extract.

The histogram analysis standard value of inhibition for both extracts was 2.2 cm, as shown in Figure 1. The heated approach is more effective at preventing the growth of *Staphylococcus aureus* than the hot & cold method is. The concentration of 100% produced the highest mean rate of inhibition for both the extract and the three other concentrations (100%, 50%, and 25%). Results showed that the *Papaver somniferum* seed hot extract had the highest zone of inhibition for *Staphylococcus aureus* at 100% (1.960.54Cm), which decreased as the extract concentration increased by 50% (1.130.38Cm) and 25% (0.8330.77Cm). At each concentration of *Papaver somniferum*'s seeds hot extract against the *Staphylococcus aureus*, the Comparison of

Means showed a statistically significant difference (p 0.05). Cold extract likewise showed the largest zone of inhibition at 100% (1.531.11Cm), 50%(1.060.61Cm), and 25%(0.800.91Cm). The Papaver somniferum seeds Cold extract showed a statistically significant difference (p 0.05) against Staphylococcus aureus at each concentration in the comparison of means. Significant differences existed between the Papaver somniferum seeds' hot vs. cold extract concentration of 100% against Staphylococcus aureus.

Extracts from the seeds of Papaver somniferum have a lot of potential as antibacterial substances against microbes. The can therefore be utilized to treat infectious diseases brought on by resistant microorganisms. The effectiveness of the seed extract from Papaver somniferum against resistant bacteria opens up a new range of infectious diseases. Plants have a big potential for creating new, highly beneficial medications for people. The search for biologically active principles in higher plants can be done in a variety of ways. The Papaver somniferum plant contains compounds such as flavonoids, alkaloids, glycosides, phenols, resins, etc. that have considerable antibacterial and anthelmintic properties (Manju, 2010). Similarly, pathic was also discovered, along with the aforementioned compounds as well as tanins, saponins, and terpinods. These compounds work quite effectively against microorganisms that resemble E. coli15. (Pathak, 2021). However, in the current investigation, hot and cold extracts of Papaver somniferum's seeds both shown significant effects at various concentrations, with the hot extract exhibiting the strongest effects. The dried poppy pollen exhibits widespread antibacterial activity, according to Ismaili, 2017. Mishra, 2021, consistently discovered that the seeds of the Papaver somniferum plant have antibacterial effect against E. coli, molds, Pseudomonas, Salmonella, and other germs. It's also

mentioned that poppy seeds were used to treat skin infections and diseases like eczema. The siddha concept states that Papaver somniferum seeds can be used to kill microorganisms and treat diarrhea, dysentery, and itching. Dysentery is undoubtedly brought on by an infection or infestation. Similar to diarrhea, itching is typically caused by infection. Therefore, Papaver somniferum's seeds can be used both internally and topically to treat this illness.

## **CONCLUSION**

The hot aqueous extract of Papaver somniferum seeds is more effective than the cold extract against Staphylococcus aureus in 2g/10ml, 100% concentration, and maximum zone of inhibition for 100% (1.960.54Cm), according to research comparing the two. Therefore, it was determined that Papaver somniferum seed aqueous extract has significant antibacterial activity against Staphylococcus aureus.

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