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EVALUATION OF ALKALOIDS EXTRACT FROM MEDICINAL PLANT *NIGELLA SATIVA* SEEDS ON PATHOGENIC BACTERIAL GROWTH

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ABSTRACT
For centuries, Nigella Sativa (NS) seeds have been utilized medicinally as both herbs and oil. A vast amount of research has been done recently on the potential interactions between these seeds' constituents and the pharmacological effects of these seeds. Several of these studies used various extracts to highlight their antimicrobial properties. In our study, we attempted to use the ground seeds to mimic the typical human digestive system. Nigella sativa seeds were tested for antibacterial activity using a modified paper disc diffusion method. At 100% concentration, there was a noticeable suppression of Pseudomonas aeruginosa and Staphylococcus aureus growth, compared to distilled water as the control. Nigella sativa ground seeds from Pseudomonas aeruginosa exhibited a greater degree of inhibition compared to Nigella sativa ground seeds from Staphylococcus aureu. As the concentration of extract increased, so did the growth inhibition. Melanin and thymoquinone, two significant active components of Nigella sativa, may be responsible for the positive inhibition. It was discovered that N. sativa extract had a very positive effect on treating the diarrhea caused by Pseudomonas aeruginosa and Staphylococcus aureus.

Keywords: Alkaloids, Nigella sativa, Pseudomonas aeruginosa and Staphylococcus aureus,

1. INTRODUCTION
An important component of cellular energy homeostasis is the enzyme MP-activated protein kinase (AMPK), and the AMPK pathway is crucial for controlling glucose and lipid metabolism. By stimulating skeletal muscle fatty acid oxidation and muscle glucose uptake, inhibiting the synthesis of cholesterol, lipogenesis, and triglycerides, and influencing pancreatic beta-cell insulin secretion, AMPK activation can affect a variety of physiological processes.

It has also been demonstrated that AMPK is a target for antidiabetic medications, such as metformin, and a number of plant-based natural products derived from conventional medicine. Black cumin, or *Nigella sativa* Linn. (Ranunculaceae), is a plant that grows in Southwest Asia, South Europe, and Mediterranean and Middle Eastern nations. For centuries, people have been consuming the seeds of *Nigella sativa*, which are also widely used as a spice and in traditional medicine to treat a variety of illnesses, including diabetes. Previous studies on the phytochemistry of *N. sativa* seeds have identified oils, saponins, flavonoids, and alkaloids.

Notably, *N. sativa* is one of only two Nigella species that have been found to contain indazole-type alkaloids across all natural sources as of this writing. The antidiabetic properties of *N. sativa* seeds have been extensively researched, and thymoquinone, the plant's most abundant oil constituent, is one of the main bioactive compounds thought to be responsible for this activity. Nevertheless, there have also been reports of antidiabetic effects from defatted and aqueous extracts of *N. sativa* seeds, which is how the previously mentioned rare indazole-type alkaloids were isolated. Additionally, it was reported that the AMPK pathway mediates the in vivo antidiabetic activity of an *N. sativa* seed extract; however, it is unknown if indazole-type alkaloids play a role in these effects. Numerous investigations and studies on the pharmacological effects of these seeds have been conducted recently. These studies have been conducted in the fields of experimental and clinical pharmacology, and they have looked into the use of these plants as well as the components and active ingredients that may be able to explain or interpret their pharmacological actions. Thymoquinone, a volatile oil found in these seeds, and Melanine, a fixed oil, are two of these active ingredients (...). Using their extracts or oil, some studies were conducted to

demonstrate the potential antimicrobial and antibacterial properties of these seeds (... The distinct anti-inflammatory properties of these seeds could be explained by the antioxidant properties of thymoquinone and its inhibition of 5-lipoxygenase. It's interesting to note that thymoquinone, the active ingredient in *N. sativa*, was found to have less of an anti-eicosanoid and antioxidant effect in fixed oil . Aim of this study was to determine the antibacterial activity of *N. sativa* extract on the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS METHOD

The following procedures were followed in order to determine the antibacterial activity of Nigella Sativa ground seeds dissolved in water using a modified disc diffusion method. The tools were thoroughly cleaned and sterilised by submerging them in ethanol for ten minutes. After that, they were rinsed with distilled water (D.W.) and sufficiently dried with sterile bandages.

After being dried and ground, Nigella sativa was placed in bottles. Using a sensitive balance, 1.0 gm of Nigella sativa was measured and then carefully transferred using a spatula into individual foil-covered test tubes. In the microbiology lab, 2.5 milliliters D.W. was added to each test tube. Methanol and chloroform were used to help with the extraction process. The agar plate was aseptically prepared by pouring sterile agar.

Conversely, nutrient broth was inoculated with a loopful of the chosen pathogenic bacteria, and the broth containing these bacteria was also inoculated onto plain agar. The same solvent used to extract the extract was used to prepare four different concentrations of the extract: 25, 50, 75, and 100%. After shocking the plan disc in the solution, it was placed on the agar plate by dividing it into four sections. In order to evaluate the outcomes, the tested plates were lastly put in an incubator set at 37ºC for a full day. Following each experiment, images of the Petri plates were captured, and the inhibition zones surrounding each paper disc (5 mm in diameter, filter paper) were measured and noted.

RESULTS AND DISCUSSION

The identification of the antibacterial effect was achieved by taking different concentration of extract of *N. sativa*. Without diluted extract was found more effective for both bacterial strains *Pseudomonas aeruginosa and Staphylococcus aureus.* T Figure 1 showed the comparison of the inhibition caused by the different concentrations of extract of *N. sativa.* The largest inhibition was observed for *Pseudomonas aeruginosa* due to the extract of N. sativa in methanol. Maximum inhibition for *Pseudomonas aeruginosa* were observed for 100% followed by 75, 50 and 25%. Similarly 100% concentration was found more effective for *Staphylococcus aureus*. But, extract of *N. sativa* from bothe showed more effect on *Pseudomonas aeruginosa* as compared to *Staphylococcus aureus* (Fig. 1 a and b). No inhibition was observed by the negative control i.e. with solvents and distilled water. Table 1 and 2 showed the inhibition zone size of extract of *N. sativa* with various solvent. Result showed the methanol was found effective solvent to extract the alkaloid of *N. sativa*. Alkloid was found antipyretic and antibacterial activity. Similar type of observation was found by various works (Yuan et al., 2014; Tiji et al., 2022). Morsi had proven that both the crude alkaloid extract and the water extract of the seeds were effective against some tested microorganisms like staphylococcus despite their resistance to other antibiotics (Ali and Blunden, 2003). Probably these variations could be explained by the different extraction methods, and also the amount of ingredients of the same plant can be affected by the area and season of collection. A combination of garlic with NS was tested individually and in combination for their antimicrobial activities against *Staphylococcus* where both showed modest antimicrobial effects (Roy et al., 2006). Zuridah et al., (2008) were also found negative effect of N.S. on the growth of *E. coli* by using methanolic extract and concentration 25mg/ml. Although the mechanism of the antimicrobial effect of these seeds has not been reported, its antimicrobial action could be attributed to the active ingredients especially thymoquinon and melanin .

CONCLUSION

According to the method used, a clear and undeniable antibacterial effect caused by the extract of *N. sativa* was found more effective for *Pseudomonas aeruginosa* as compared to *Staphylococcus aureus.* Among solvent

methanol showed effective for extraction as compared to chloroform. From this study it was concluded that extract of *N. sativa* may be effective for disease caused by variou bacteria.

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Table 1: Inhibition zone due to various concentration of extract of *N. sativa* obtain by methanol on *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Various concentration of extract of *N. sativa* (%)	**Size of inhibition on *Pseudomonas aeruginosa***	**Size of inhibition on *Staphylococcus aureus***
Control (Distilled water)	0 mm	0 mm
25	10 mm	6 mm
50	15 mm	8 mm
75	15 mm	8 mm
100	25 mm	10 mm

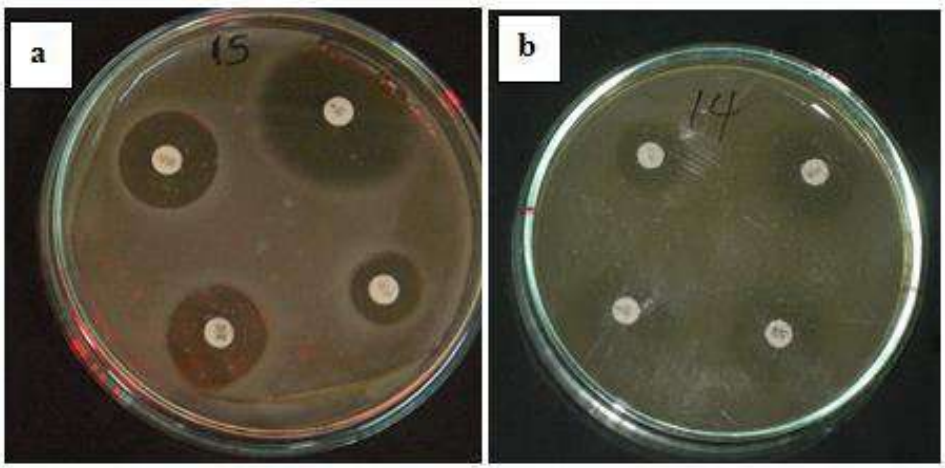

Fig. 1: Showing inhibition effect of extract of *N. sativa* due to methanol on *Pseudomonas aeruginosa* (a) and *Staphylococcus aureus (b)*

Table 2 : Inhibition zone due to various concentration of extract of *N. sativa* obtain by chloroform on *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Various concentration of extract of *N. sativa* (%)	**Size of inhibition on *Pseudomonas aeruginosa***	**Size of inhibition on *Staphylococcus aureus***
Control (Distilled water)	0 mm	0 mm
25	8 mm	4 mm
50	13 mm	6 mm
75	18 mm	7 mm
100	20 mm	8mm