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BACTERICIDAL POTENTIAL OF SELECTED MEDICINAL PLANTS AGAINST STAPHYLOCOCCUS AUREUS ISOLATED FROM FISH

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ABSTRACT

Approximately 7.2 billion people around the world consume 136.2 million tons of fish annually. The need for fish meat and proteins intake increases day by day because most of the human population fulfils their proteins deficiency using fish meat and fish proteins. As fish are living in water therefore, they are directly exposed to many pathogenic microorganisms inhabiting the water environments of farms or in wild conditions. A Gram-positive bacterium, S. aureus is the major cause of numerous infections in a wide range living things and food poisoning. For thousands of years, the seeds of N. sativa are traditionally used for their medicinal importance particularly for the cure of infectious diseases and as health promoter into the animal feed. In the current study, identified cultured strains of S. aureus were obtained and invitro antibacterial activity of aqueous extracts of Nigella sativa, Aloe barbadensis, Curcuma longa, Calotropis procera, and Kalanchoe daigremontiana was evaluated. In this study it was concluded that N sativa, A. barbadensis, C. procera and K. daigremontiana exhibit antibacterial potential against S. aureus isolated from fish. It is also revealed that both N. sativa, and A. barbadensis exhibit a remarkable antibiotic potential that is quite comparable to the modern antibiotic (Azithromycin).

INTRODUCTION

A significant source of protein for humans is fish. Approximately 7.2 billion people around the world consume 136.2 million tons of fish per year (Andrew, 2001; FAO, 2014). This sector provide employment to about 600,000 people of Pakistan (FAO, 2012). Fish are exposed to many pathogenic microorganisms inhabiting the water environments of farms or in wild conditions (Bondad-Reantaso et al., 2005). About 30% of landed fish are lost through microbial activity (WHO, 2007). A Gram-positive bacterium, S. aureus is the major cause of numerous infections in a wide range living things and food poisoning (Licitra et al., 2013). It is the major cause of diseases in fish like skin darkness, loss of scales, skin ulcers and hemorrhages on gills and fins, swellings on eyes and abdomen (Saint, 1996). Plants are the major

sources for many bioactive compounds that can be utilized for treating different diseases in multiple organisms. For thousands of years, the seeds of *N. sativa* have been traditionally used for their medicinal importance particularly for the cure of infectious diseases and as health promoter into the animal feed (Alam, 2010). Due to exposure of antibiotics the genetic adaptation of microorganisms leads to antibiotic resistance. Moreover, the available drugs are cost effective. That is why the emergence of multi-drug-resistant strains requires new therapeutic strategies (Knöppel et al., 2017). S. aureus is among the major pathogens which are responsible for causing many diseases in fish. Very less published data is available about the therapeutic use of the selected plants against S. aureus. Therefore, the present research study aims to assess the antibacterial potential of the selected plants against S. aureus.

Materials and Methods

Ethical Approval

Ethical consideration was obtained from Research Ethical Committee, KUST Kohat.

Isolation of Bacteria:

Identified cultured strains of S. aureus were obtained from the Microbiology laboratory of the Department of Microbiology, KUST, which was isolated from fish.

Plants collection

Leaves of Kalanchoe daigremontiana, Calotropis procera and Aloe barbadensis were collected from their natural habitats in District Kohat and seed of Nigella sativa and tubers of Curcuma longa were purchased from local market to evaluate their antibacterial properties against S. aureus. They were identified by experts of the Botany Department, KUST and were transported to Microbiology Laboratory of Kohat University of Science and Technology, Kohat for further processing.

Extract Preparation

The parts of plants were dried in shade for few days, then grinded and 20-25g of each of the grinded material was soaked in 0.5 liters of distilled water. The gel of Aloe barbadensis was also extracted from leaves and mixed in 0.5 liters of distilled water. All mixtures were placed in shaker for 5-7 days at room temperature. The mixtures were then filtered, leaving at room temperature to evaporate the solvent. The extracts were then diluted with distilled water to prepare the stock solutions to obtain the final concentrations of 0.8 gm/ml of each plant extract (Nanasombat & Lohasupthawee 2005).

In Vitro Assay

The antibacterial in-vitro activity of *N. sativa* was evaluated by using agar well diffusion method as described earlier (Gupta et al, 2008). Azithromycin antimicrobial was used as standard positive control in this study. A cotton swab was used to evenly streak the bacterial broth culture on sterile Muller-Hinton agar (MHA) plates to form a bacterial lawn. After half an hour, sterile cork borer wells were created at equal intervals on each plate. 100μL were put into the wells with labels of each test extract. For comparison, Azithromycin (0.8 gm/mL) was used as a positive control, respectively. After that, the plates were allowed to rest for an hour so that the extracts could properly diffuse into the media. The plates were then incubated for 24 hours at 37 °C. Following incubation, a ruler was used to measure the ensuing zones of inhibition's dimensions, which were then expressed in millimeters (mm). All the plants which were used during this research have been given in Table 01.

Table 01. Shows the detail of plants that were used during the research.

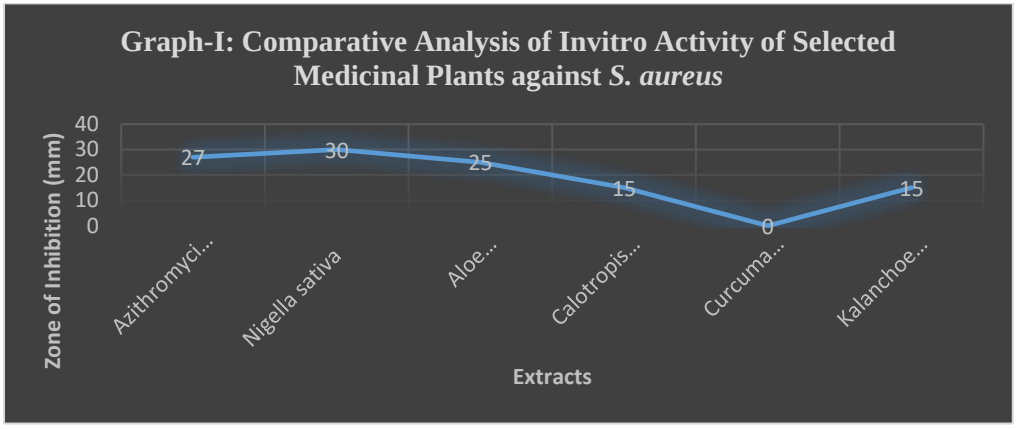
Scientific Names	English Name	Local name	Part Used	Extract Type
Nigella sativa	Kalwanjee	Kalwanjee	seeds	Aqueous
Aloe barbadensis	Aloe Vera	Karghandal	Gel	Aqueous
Curcuma longa	Turmeric	Haldi/korkamand	Tuber	Aqueous
Calotropis procera	Milkweeds	Akh	Leaves	Aqueous
Kalanchoe daigremontiana	Mother of thousands	Zakhm-e-hayaat	Leaves	Aqueous

Results

A clear zone of inhibition was observed in Sample no.1 (Azithromycin) with the inhibition zone of 27mm. Azithromycin was used as positive control in this study. It was observed that Nigella sativa revealed a remarkable antibacterial activity against S. aureus, which was considered quite comparable with the positive control. The inhibition zone of N. sativa was about 30 mm, but the edges of the zones were bit blurred. Aloe barbadensis extract also revealed good antibiotic activity with the inhibition zone of 25 mm. Almost same antibacterial activity was observed by Calotropis procera and Kalanchoe daigremontiana extracts with the inhibition one of 15 mm each. In this study no antibacterial activity of Curcuma longa was observed. The zones of inhibition have been given in Table-02 and Graph-01. The in-vitro activity of the selected medicinal plants is shown in Figure-1

Table 02. Represents the zones of inhibitions of the plant extracts.

S. No.	Extract	Concentration gm/ml	Zone of Inhibition (mm)
1	Azithromycin (Control)	0.8	27
2	*Nigella sativa*	0.8	30
3	*Aloe barbadensis*	0.8	25
4	*Calotropis procera*	0.8	15
5	*Curcuma longa*	0.8	0
6	*Kalanchoe daigremontiana*	0.8	15



Graph-1; Presentation of zones of inhibition of aqueous extracts of selected medicinal plants.

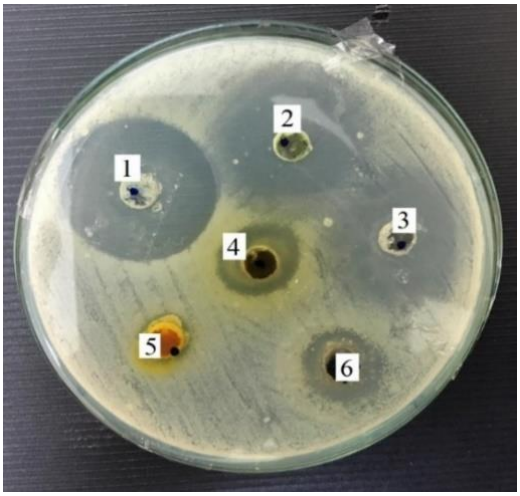


Figure-1; In vitro activity of selected medicinal plants against S. aureus.

Discussions

Among the tested plant extracts, *Nigella sativa* extract has shown the best antibacterial activity in aqueous solvent. The finding of Nigella sativa extract agrees with the work of Mukhtar and Ghori, 2012. The aqueous extract of C. procera also proved effective against S. aureus which coincides with the study of (Mako et al., 2012. C. longa exhibits antibiotic potential as per study of (Mukhtar and Ghori, 2012) but in this study, it didn't show any antibacterial activity, this might be due to low quality sample of C. longa. A. barbadensis extract also showed antibacterial activity against S. aureus and its result shows similarity with the study of (Kumari and Gupta, 2015). K. daigremontiana aqueous extract also showed antibacterial activity against S. aureus.

Conclusion

In this study it was concluded that *Nigella sativa*, *A. barbadensis, C. procera* and K. daigremontiana exhibit antibacterial potential against *S. aureus* of fish. It is also revealed that both *Nigella sativa*, and A. barbadensis exhibit a remarkable antibiotic potential that is quite comparable to the modern antibiotic (Azithromycin). The unclear edges of zone of inhibition of both *N. sativa* and *A. barbadensis* might decrease in the antibiotic potential with the passage of time.

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