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Antibacterial activity of *Rosa damascena* petal extracts against the fish pathogen *Aeromonas hydrophila*

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ABSTRACT

There has been an increase in the emergence of antibiotic resistance in pathogenic bacteria which has led us to discover alternative methods to eliminate bacterial infections. In the present study an attempt has been made to determine the antibacterial activity of aqueous, ethanol, methanol and chloroform extracts of Rosa damascena petals extracts against the fish pathogen Aeromonas hydrophila by disc diffusion method. The results revealed that aqueous extract of R. damascena petals showed highest antibacterial activity against the fish pathogen A. hydrophila at 100, 200 and 300 ppm concentrations followed by methanol, ethanol and chloroform. Compounds have been identified within herbal plants, such as roses serve as alternatives to antibiotics and are source for the production of phytochemical compounds that are effective against antibiotic-resistant strains of bacteria.

Key words: Herbals, antimicrobial activity, fish pathogen, disc diffusion.

INTRODUCTION

Bacterial infections are the major reasons for fish mortality in aquaculture industry (Naylor and Burke 2005) [20]. These outbreaks are mainly due to the evolution of multidrug resistant microbes. The increase in antibiotic resistant strains of clinically important pathogens has led to the emergence of new bacterial strains that are multi-resistant [31; 4] The need for alternative drugs to save the fishes from pathogenic infections, which leads to increased mortality in fish farms and in turn affect the economic growth has become essential.

Aeromonas hydrophila is a gram negative opportunist bacterium associated with aquatic animal disease [7]. *A. hydrophila* causes mass mortalities in several species including Carps, Snake heads, Gouramies and Cat fishes and are considered as an etiological agent of several diseases such as emaciation, haemorrhagic septicaemia, asymptomatic septicaemia, ulcerative infection, tail rot and fin rot [22]. It also causes Motile Aeromonas Septicaemia (MAS) and Epizootic Ulcerative Syndrome (EUS) as a primary pathogen [26]. Herbs and herbal products play a significant role in fish culture [23]. The ability of herbs to inhibit bacterial activity of fish pathogens have been well documented [10, 19, 3, 9, 6 & 11].

Roses are woody perennial plants of the genus *Rosa* within the family Rosaceae. They are erect shrubs with sharp prickles. Acetone and aqueous extract of *R. damascene* petals have the potential of antimicrobial activity against *E. coli* and *B. subtilis* [18]. Strong antibacterial activity against several bacteria such as *E. coli, Pseudomonas aeruginosa, B. subtilis, Staphylocccus aureus, Chromobacterium violaceum* and *Erwinia carotovora* strains have been reported for *R. damascena* essential oil and absolute [29]. Fresh flower and spent flower extracts of *R. damascena* has been reported to exhibit antibacterial activity [21]. The present study aims at finding out antimicrobial activity of aqueous, ethanol, methanol and chloroform extracts of *R. damascena* petals against the fish pathogen *A. hydrophila*.

MATERIALS AND METHODS

Preparation of Rose petals extract

Shade dried and coarsely powdered petals of *R. damascena* were extracted with four different solvents such as water, ethanol, methanol and chloroform. 50 grams of rose petals was extracted in 100ml of each solvent, allowed to stand for one week with frequent shaking. After one week the solvents were filtered and evaporated, the residues were collected and 10 mg of each residue was dissolved in 10 ml of distilled water. From the stock solution 100 µl was taken and made upto 1 ml containing 100 µg or 100ppm of extract. Similarly concentrations of rose petal extracts containing 200 and 300 ppm were prepared.

Test microorganism - *Aeromonas hydrophila*

Aeromonas hydrophila–MTCC1739 was supplied by the Institute of Microbial Technology, Chandigarh. Lyophilized cells of *A. hydrophila* were sub-cultured and maintained in nutrient agar broth at 4˚C in the refrigerator. All the experiments were performed under strict aseptic conditions.

Disc diffusion method

The antimicrobial assay was performed by Bauer *et al.* [8]. Muller-Hinton Agar was prepared and sterilized. The media was allowed to cool to hand bearable temperature and about 20ml was poured into petriplates (12x12cm) and kept for solidification. After solidification, *A. hydrophila* broth culture were swabbed over the media. Sterilized Whatman filter paper disc (Himedia – 6mm diameter) were impregnated with different volumes of (100, 200 and 300 ppm) *R. damascena* petal extracts, placed over the media and kept for incubation at 37˚C for 24 h. Tetracycline loaded disc were maintained as positive control and distilled water loaded disc as negative control. The discs were placed with adequate space in-between so as to prevent merging of inhibition zones. Each disc was pressed firmly to ensure complete contact on agar surface. Triplicates were maintained for all the experiments. The results were observed the next day and the zone of inhibition level was measured using a zone measuring scale.

RESULTS

Aqueous extract of *R. damascena* petals showed highest antibacterial activity against the fish pathogen *A. hydrophila* with the inhibition zone of 17.33 ± 1.15 at 100ppm, 16.33 ± 0.58 at 200ppm and 16.0 ± 1.0mm at 300 ppm of rose petal extract respectively. Ethanolic extract of *R. damascena* petals showed inhibition zone of 14.67 ± 0.58, 15.67 ± 0.58 and 15.0 ± 0.0 mm at 100, 200 and 300ppm respectively. Methanol extract showed inhibition zone of 15.33 ± 0.58, 15.67 ± 0.58 and 15.0 ± 0.0 mm at 100, 200 and 300ppm of rose petal extract respectively. Similarly chloroform extract of *R. damascena* petals extract showed inhibition zone of 13.33 ± 1.15, 15.67 ± 0.58 and 14.67 ± 0.58 mm at 100, 200 and 300ppm respectively. Thus, of the various extracts of *R. damascena* petals, aqueous extracts showed maximum inhibition zone at the lowest concentration of 100 ppm. Ethanol, methanol and chloroform extracts showed highest activity of 15.67 ± 0.58 at 200 ppm concentration. At 100 and 300ppm the inhibition zone was lesser ranging from 13.33 ± 1.15 to a maximum of 15.67 ± 0.58mm. The positive control Tetracyclin showed inhibition zone of 28mm (0.1mg/disc). In distilled water, used as negative control there was no inhibition zone.

DISCUSSION

R. damascena extracts possess moderate broad spectrum antimicrobial activity against gram positive, gram negative, acid fast bacteria and fungi. Gram positive bacteria were inhibited by a concentration ranging from 0.125-2mg/ml of extracts and killed by 0.5-4mg/ml. Gram negative bacteria were inhibited by concentrations ranging between 2-8 mg/ml and killed by 4 to >8mg/ml extract. *A. haumannii* has been reported to be the most sensitive gram-negative bacterium and was inhibited by 1to 4mg/ml and killed by 2 to 8mg/ml. *K. pneumonia* was the least sensitive one [27].

Antibacterial activity of *R. damascena against E. coli, B. subtilis* has been reported by Kurhade *et al.* [18]. Aqueous extract of rose petals exhibits antibacterial activity, suggesting a possible utilization of rose petal boiling water after rose oil distillation. Hot water and ethanolic extract of *R. damascena* inhibited growth of *S. aureus* up to 18mm and 34mm respectively [2]. Similar to the present study aqueous extract of *Azardirachta indica* showed inhibition zone of 18mm against *A. hydrophila. Solanum torvum* demonstrated moderate (11mm) and *Curcuma longa* marked weak (8mm) inhibition against *A. hydrophila* [3]. Existence of variations in antibacterial activity among various Rosa taxa fruits against a number of bacteria have been reported and *R. canina* were found to be more active on microorganisms [28].

Water, ethanol, methanol and hexane extracts of *R. damascena* have been reported to inhibit the growth of *Acinetobacter calcaoceuticus, E. coli, Pseudomonas aeruginosa, Streptococcus pneumonia, Staphylococcus aureus* and *Aspergillus niger* [13]. Of all the above extracts, aqueous extracts showed highly significant antibacterial activity and the zone of inhibition ranged from 13-28mm, while ethanol extract showed inhibition which ranged between 15mm for *A. niger* to 34mm for *P. aeruginosa*. Methanolic extracts showed moderate activity ranging from 13mmto 25mm. 300µl of methanolic extract of *R. damascena* showed maximum activity (25 ± 1.632mm) followed by 200 µl of extract (23.66 ± 1.246mm) and 100µl extract showed moderate activity against the dental pathogen *Streptococcus mutans* [30]. Petroleum ether extract of rose is reported to show maximum of 29mm of inhibition against *P. aeruginosa* as compared to *E. coli* with 16mm, *S. pneumonia*, 16mm, *Salmonella typhimurium*, 15mm, *Enterobacter aerogens*, 16mm, *Proteus vugaris, Staphylococcus aureus, S. epidermidis, Bacillus subtilis* and *Citrobacter frundii* with 15mm zone of inhibition [14].

Similar to rose petals, *Hibiscus* petals are also reported to possess antibacterial activity. 300µl of ethanolic extract showed maximum activity (23 ± 1.632mm) followed by 200µl of extracts (20.66 ± 1.246) and 100µl extract showed moderate activity against the dental pathogen *Streptococcus mutans* [30]. Chloroform extracts of flowers of *Aerva lanata* showed inhibitory activity against *A. hydrophila, V. alginolyticus* and *E. coli* with inhibition zone of about 23, 23 and 26 mm respectively [16]. The concentration of the extract seems to influence the inhibitory activity where the zone of inhibition was 16.33, 21.33 and 23.33mm with 50, 75 and 100µl concentration. However in the present study, the concentration of the extract does not appear to influence the inhibitory activity.

Similar antimicrobial potential has been reported with flower extracts of the plant *Couroupita gauanensis* against *V. alginolyticus* and *Plesiomonas shigelloides* (Ramalakshmi *et al.*, 2013) [24]. Flower extracts of *Achillea falcate* are also reported to show antibacterial activity against *A. hydrophila* (Al Lahmam and Al Fadel, 2014) [5]. Methanol extracts of *Bryophyllum pinnatum* and *Kalanchoe crenata* were most active against *Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis* and *Pseudomonas aeroginosa* [15]. Methanol extracts of *Boerhaavia diffusa, Tinospora cardioflora* and *Eclipta alba* leaves have been reported to show significant *in-vitro* antimicrobial activity [12]. The hydrophobic nature of plant extracts enables them to partition lipids of the bacterial cell membrane and mitochondria, disturbing cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions leads to death [25]. The inhibitory effect may be also due to the presence of tannin through producing hydrogen bonds with proteins, which converts its structure and blocks protein synthesis [17].

Table. 1 Antibacterial activity of different extracts of *R. damascena* petals against *A. hydrophila*

***R. damascena* extracts**	**Diameter of zone of inhibition (mm)**		
Extract	100 µg/ disc	200 µg/disc	300 µg/disc
Aqueous	17.33 ± 1.15	16.33 ± 0.58	16.0 ± 1.0
Ethanol	14.67 ± 0.58	15.67 ± 0.58	15.0 ± 0.0
Methanol	15.33 ± 0.58	15.67 ± 0.58	15.0 ± 0.0
Chloroform	13.33 ± 1.15	15.67 ± 0.58	14.67 ± 0.58
Tetracycline (Positive control)	2.8 ± 0.0		
Distilled water (Negative control)	-		

CONCLUSION

Plant derived medicines provide a cheaper source for treatment and greater accuracy than chemotherapeutic agents. Medicinal plants as alternative agents are effective to treat infectious diseases and mitigate the side effects associated with synthetic antimicrobials. From the above study it is clear that *R. damascena* petals extracts are capable of inhibiting *A. hydrophila.* The application of herbal extracts can decrease the use of antimicrobial agents and have excellent potential in improving aquaculture.

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