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EVALUATION OF ANTI-QUORUM SENSING PROPERTIES OF EXTRACTS OF TRIDAX PROCUMBENS AGAINST POTENT PATHOGENS PROTEUS MIRABILIS AND SERRATIA MARCESCENS

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ABSTRACT
Medicinal plants play an important role in the development of new drugs and potent therapeutic agents. *Tridax procumbens*, a widespread weed, is used as an important medicinal plant in Ayurveda. In the current scenario, several pathogenic organisms have become multidrug resistant due to indiscriminate use of antibiotics. There is a need to find out an alternative treatment strategy that will only inhibit the virulence of pathogens which is controlled by a phenomenon called "Quorum sensing" [QS] without challenging basic cell function so that they do not develop the resistance against antibiotics. The present study was therefore conducted to evaluate the anti-quorum sensing potential of the weed *Tridax procumbens*. Extracts of the plant were prepared in Soxhlet apparatus using solvents with increasing polarity. The extracts were tested for anti-swarming potential in *Proteus mirabilis* and pigment inhibition in *Serratia marcescens.* Petroleum ether and chloroform extracts were found to be the most potent inhibitors of quorum sensing in *Proteus mirabilis* showing 14% and 37.5% reduction in the diameter of the swarm zones and the number of the swarm rings also was significantly reduced. Dose dependent pigment inhibition in *Serratia marcescens* was observed in the presence of ethyl acetate and methanol extract with diameter of zones of pigment inhibition ranging from 14-31 mm. Results of the study indicate that the plant *Tridax procumbens* possess medicinal properties and can be studied further as a potent inhibitor of quorum sensing dependent virulence in pathogenic organisms.

KEYWORDS: Quorum sensing, Soxhlet apparatus, *Serratia marcescens*, Pigment inhibition, Swarming inhibition.

1. INTRODUCTION
Tridax procumbens is abundantly distributed weed which belongs to family *Asteraceae.* It is commonly known as coatbuttons or *Tridax* daisy. It is native to tropical America and has also been found throughout India particularly in Maharashtra, Madhya Pradesh, and Chhattisgarh regions as a weed.[1] It is also listed as a noxious weed in United states.[2,3]

Tridax procumbens has been used in India in traditional Ayurvedic medicines. It is dispensed in 'Bhringraj' which is a well-known ayurvedic medicine for liver disorders.[4] The extracts of the plant have been extensively used for wide variety of ailments. The plant is commonly used in traditional medicine as anti-coagulant, anti-fungal, for wound healing, and in the treatment of diarrhea.[5] All the parts of the plant have been studied for various medicinal properties. Leaves are used for the treatment of hairfall, dysentery and diarrhea.[6] Flowers of the plant have been shown to possess anti-microbial properties. The flower extracts have been shown to possess good bactericidal properties against pathogens like *E. coli* and *S. aureus*.[7] The stem and whole plant extracts have also been shown to possess anti-bacterial properties against *E.coli*, *P.mirabilis* and *K. pneumoniae*.[8] In one study it was observed that the plant extract possesses bactericidal activities against several waterborne pathogens which are responsible for various diseases like diarrhea, cholera, salmonellosis etc.[9] The methanolic extract of leaves of *Tridax procumbens* showed antioxidant potential indicating that various metabolites such as tannins, phenolic compounds, and flavonoids are active antioxidant compounds.[10] Leaf extracts were found out to be bactericidal against multidrug resistant and nosocomial pathogen like *Pseudomonas aeruginosa*.[11] Chloroform extract of aerial parts of *Tridax* possesses hepatoprotective property. This study suggested that the weed can be a multi-useful plant which has got many medicinal uses for various ailments.[12]

Many of the previous studies were done to evaluate the antifungal, antimicrobial, immunosuppressive and antitumor activities of *Tridax procumbens.* However

anti-QS property of this plant has not been previously reported. Therefore, the present study was carried out to evaluate the quorum inhibition activity of the weed. Development of multidrug resistance in bacteria is becoming global concern these days. Many of the pathogenic organisms are developing multidrug resistance due to indiscriminate use of antibiotics. This becomes a problem in the treatment of various infectious diseases. Therefore, there is a need to find out an alternative strategy and inhibiting QS can be a promising way to combat these pathogens. The expression of the virulence genes in pathogens is generally QS controlled.[13] So, if QS is inhibited these pathogens can be rendered avirulent making them unable to establish disease. Since the cell viability is not affected, the chances of developing resistance against anti-QS agents are minimal.

In the present study the anti-QS potential of the *Tridax procumbens* was evaluated against two pathogens *Proteus mirabilis* and *Serratia marcescens*.

2. MATERIALS AND METHODS

2.1 Collection of plant material

Plant was collected from the nearby premises. Leaves of the plants were air dried in the shade for 5-6 days. Once dried, the leaves were ground and extracted using a Soxhlet apparatus with solvents in the series of increasing polarity- petroleum ether, chloroform, acetone, ethyl acetate and methanol. The solvents were evaporated to dryness and further dilutions were made in the DMSO to obtain the required extract concentration for different assays. For all the assays the extracts were concentrated to 10 mg/ml and further dilutions were made to get the required concentration.

2.2 Bacterial strains

Two pathogenic strains were utilized in the study *Proteus mirabilis* and *Serratia marcescens. Proteus mirabilis* a pathogenic strain which shows swarming motility pattern that is controlled by quorum sensing and *Serratia marcescens*, again a pathogenic strain which shows pigment producing capability that is also quorum sensing controlled. Both the strains were grown in LB broth and nutrient agar medium at temperature ranging from 30° to 37°C.

2.3 Bioassay for anti-quorum sensing

Swarming motility assay and pigment inhibition assay were selected for testing of anti-quorum sensing potential of the plant extracts.[14,16]

2.3.1 Swarming motility assay (*Proteus mirabilis*)

Swarming motility assay was conducted with LB media consisting of 1.5% agar. [15] Swarm plates were poured and allowed to dry at 37°C before being used. Swarm plates with different concentrations of the extracts were then centrally inoculated with 5 µl of the overnight grown *Proteus mirabilis* culture in LB broth. Swarm plates containing the same amounts of DMSO were used as control were similarly centrally inoculated. These plated were then incubated for 24 hrs at 37°C. Inhibition in the swarming motility was determined by counting the number of swarm rings and measuring the diameters of the swarm zones as compared to control.

2.3.2 Pigment inhibition assay (*Serratia marcescens*)

Agar well diffusion assay was adopted to detect pigment inhibition in chromogenic bacterium *Serratia marcescens.* The assay was conducted with nutrient agar medium consisting of 2% agar. The nutrient agar plates were poured with molten agar containing 1 ml of bacterial culture and allowed to solidify. Once completely solidified, wells were made using well borer. Then appropriate volumes of DMSO (control) and plant extracts were loaded into each well according to increasing concentration. Plates were incubated for 24 hrs to check the inhibition of pigment production around the well.

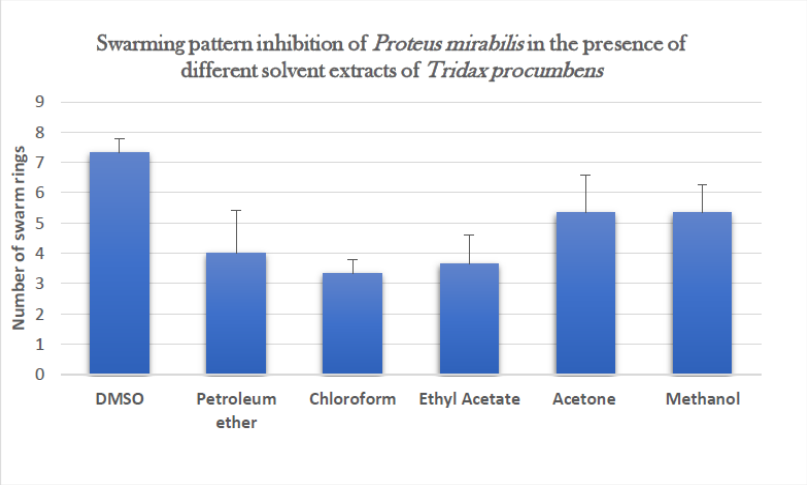
3. RESULTS

3.1 Anti swarming assay

All the five extracts viz petroleum ether, chloroform, ethyl acetate, acetone and methanol were tested for the anti-swarming activity against *Proteus mirabilis.* Any change in the swarming pattern is considered as inhibition in quorum sensing mechanism.[17,18] The results of the assay were interpreted in terms of reduction in the number of swarm rings as shown in Graph. 1 and reduction in the diameter of swarm zones as compared to control plate as shown in Graph 2. Out of the five extracts tested, petroleum ether (4±1.41), chloroform (3.66±0.94) and ethyl acetate extract (3.66±0.94) showed maximum inhibition in the number of swarm rings. However maximum inhibition in the swarm zone diameter was observed in the presence of petroleum ether extract (62.33 ± 0.20) and chloroform extract (45.66± 0.09) as compared to swarm zone diameter in control (72.33±0.20). Thus there is a marked reduction in not only the number of the swarm rings but also the overall diameter of the swarm zone in the presence of chloroform and petroleum ether extracts as shown in Fig. 3.

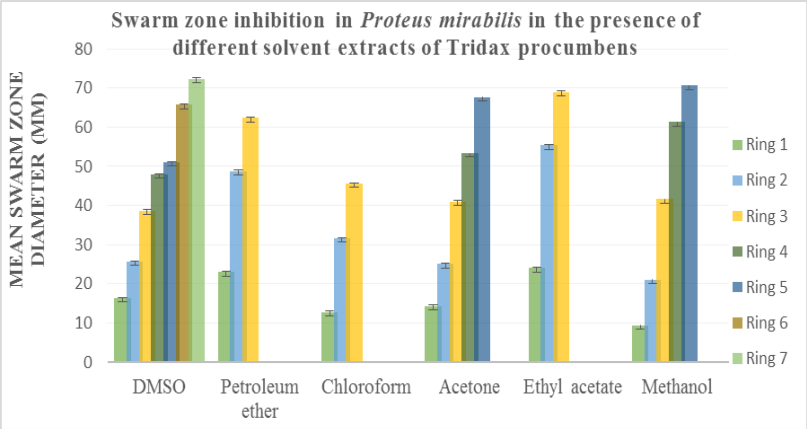
3.2 Pigment inhibition assay

To further explore the anti-quorum sensing properties of the extracts, pigment inhibition assay was adopted.[14,19] *Serratia marcescens* was used as a reporter organism.[19] This is a potent pathogen and produces a pigment called prodigiosin. This pigment production is under quorum sensing controlling genes.[20] To evaluate the extracts for pigment inhibition, agar well diffusion assay was performed. Out of the five extracts tested, ethyl acetate and methanol extracts were the most potent in inhibition pigment production. Ethyl acetate extract exhibited maximum inhibition with zone diameter of 31.66±0.47 while methanol extract exhibited maximum zone inhibition of 28±0.81 at volume of 60 µl. The pigment inhibition was also observed to be dose dependent as shown in Fig. 4 and 5.



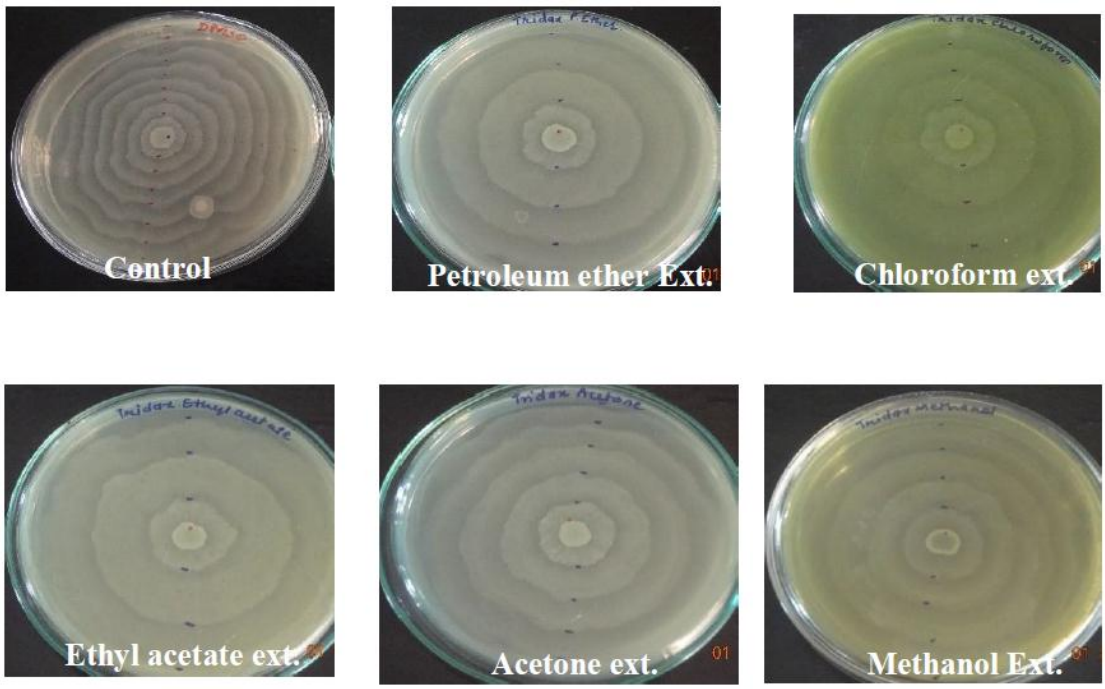
(Values are represented as mean ±SD for three experiments

Fig. 1: Decrease in number of swarm rings in the presence of different solvent extracts.



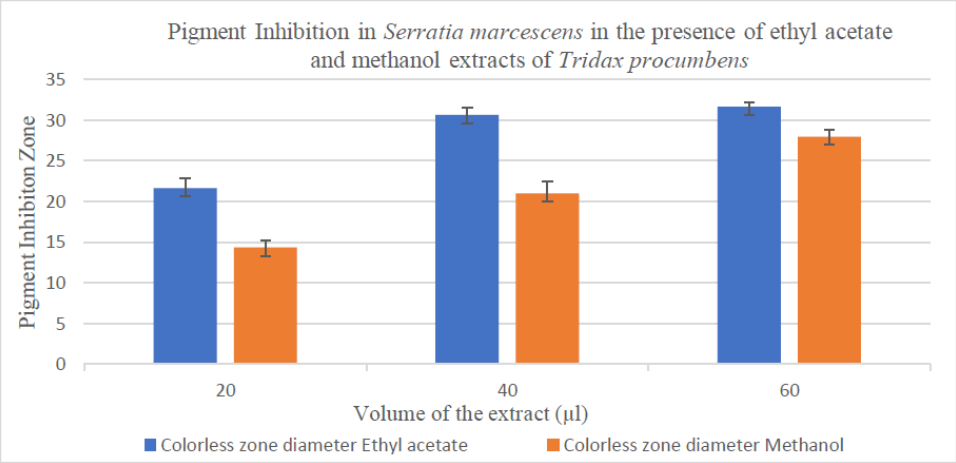
(Values are represented as mean ±SD for three experiments)

Fig. 2: Graph showing significant reduction in the swarm zone diameters in *proteus mirabilis.*



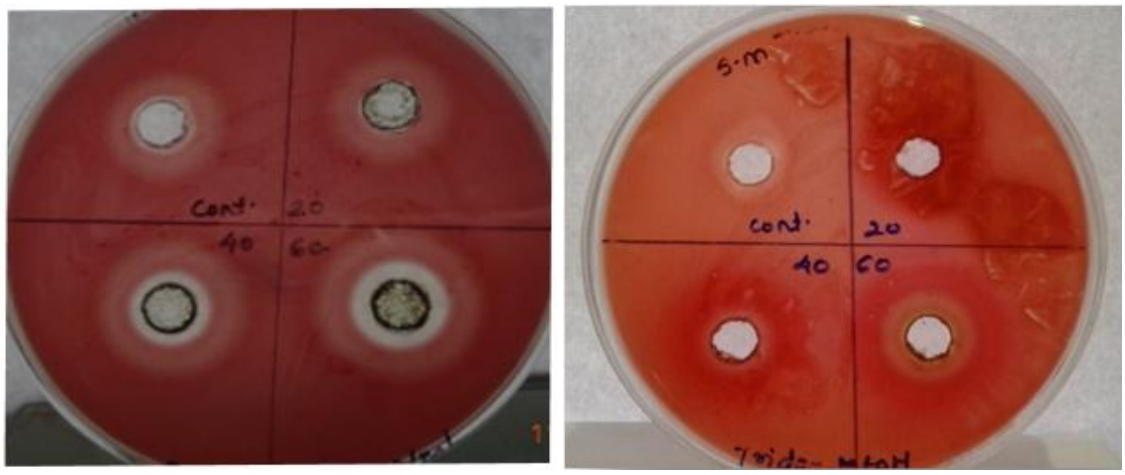
(Values are represented as mean ±SD for three experiments)

Fig. 3: Anti-swarming activity of different solvent extracts against *Proteus mirabilis.*



(Values are represented as mean ±SD for three experiments)

Fig. 4: Pigment inhibition with increasing concentration in *Serratia marcescens.*



A **B**

Fig. 5: Pigment inhibition in *Serratia marcescens* in the presence of A: Ethyl acetate and B: Methanol extracts.

4. DISCUSSION

Resistance to antibiotics in various pathogenic organisms has resulted in failure of the treatment of numerous infectious diseases. There is a need to find out an alternative strategy to combat these pathogens. With the recent technological advances isolation and purification of active lead compounds from medicinal plants has gained importance as these may provide excellent source of active compounds with potential to inhibit these multidrug resistant pathogens.[27] In this context, *Tridax procumbens* appears to be a very promising medicinal plant which contain many bioactive components with proven bioactivities. Substantial research has been carried out on the traditional medicinal properties of *Tridax procumbens*. The ethyl acetate extract of the plant showed effective inhibitory effect against *S. aureus*.[21] The plant also has been studied as a part of poly herbal preparation used in wound healing, where dose-dependent improvement in cell proliferation and angiogenesis at the site of injury was observed.[22,23] The flavonoid and saponin fraction of the plant was found to modulate the both cell mediated and humoral components of the immune system indicating that the plant possess immunomodulatory activity also.[24] The antibiofilm activity of the purified peptides from *Tridax procumbens* was evaluated and were found to be effective against *Staphylococcus aureus*.[25] The methanolic extract of the leaves of this plant was found out to be having anti-oxidant potential. Cytotoxic activity of the leaf extract of the plant also showed promising results on human lung cancer cells.[26] The plant thus possesses promising bioactivities, however very few studies have been conducted with respect to the anti-QS properties of this plant. Quorum inhibition in pathogenic bacteria can be the alternative to conventional antibiotic mediated killing which often leads to development of multidrug resistance.[27] Therefore, the present investigation focused on studying the quorum inhibitory properties of the plant. In most of the studies *Chromobacterium violaceum* is the indicator organism that is used in quorum sensing assays. But in this study *Serratia marcescens* strain is used as an indicator of quorum sensing regulation which makes the study novel. Significant quorum inhibitory properties of *Tridax procumbens* extracts were observed against potent pathogens *Proteus mirabilis* and *Serratia marcescens*.

5. CONCLUSION

It is evident from the present study that various extracts of the plant *Tridax procumbens* showed inhibitory activity against tested pathogens. Significant anti-swarming properties of *Tridax procumbens* petroleum ether, chloroform and ethyl acetate extracts were also observed against potent pathogen *Proteus mirabilis*. (Fig. 3) These extracts showed significant reduction in the diameter of the swarm zone as well as reduction in the number of swarm rings in *Proteus mirabilis.* We have observed that ethyl acetate and methanol extract at sub lethal concentration exhibits anti-QS properties in pathogen *Serratia marcescens* as indicated by the turbid zones (Fig. 5) where the cells are devoid of pigmentation. These results are indicative that plant possess anti-quorum sensing potential. The swarming motility and pigment production are the properties controlled by quorum regulatory genes in the tested pathogenic bacteria. So, inhibition of these properties indicate that the quorum sensing mechanisms are inhibited which in turn renders the bacteria avirulent without the development of resistance in these organisms.[28] The present study thus clearly highlights the anti-QS potential of *Tridax procumbens* which can be used as an alternative strategy over conventional antibiotic treatments. The plant can therefore be further explored for various combinatorial approaches for the purification and validation of the active components having potent anti QS potential.

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