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A COMPARATIVE STUDY FOR ANTIMICROBIAL PROPERTIES OF PLANTS GROWING IN NON-POLLUTED AND TANNERY POLLUTED AREAS

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ABSTRACT

It is well known that the extract from plants exhibit various medicinal benefits which have been adopted in various fields of health and medicine. In this study the antimicrobial activity of *Azadirachta indica*, *Eucalyptus sp*. and *Lantana camara* was determined against *E.coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and a comparison was made between the extract of plants grown on normal land and the one growing in tannery polluted areas. The extraction methods used for plant leaf and bark were aqueous extraction and methanol extraction. The bacterial culture were grown in brain heart infusion liquid medium at 37°C and was inoculated in agar plates. Screening for antibacterial potential of the plant extracts was done by conducting MIC (minimal inhibitory concentration) test. The methanolic and aqueous extract both showed different antimicrobial activity in terms of zone of inhibition and also the extract from the plants of tannery polluted area varied in their antimicrobial potential from that of plants grown on normal land. Although methanolic extracts were found to be more effective against microbes in comparison with aqueous extract but all the bacteria found to be inhibited though to varying degrees.

Keyword: antimicrobial, extraction, minimal inhibitory concentration

INTRODUCTION

It is an undeniable fact that plants are an ample source of various products which are used at a large scale for human beneficiary. In addition, plants are also plentiful in antimicrobial bio reactive molecules. Approximately 80% of the human population in the world is using plant based medication. Even though medicinal herbs are often advertised as natural and therefore harmless but they can produce adverse effects too, including toxic effects, allergic reactions, and drug interactions (K. Chan, 2003). Herbs such as Aloevera, *Valeriana officinalis*, and *Symphytum officinale* could also be hepatotoxic (F. Stickel*et al.*, 2005). Plants are exuberant in various types of phytochemicals such as flavonoids, terpenoids, tenants, alkaloids which are widely used as antimicrobial agents. These phytochemicals can be extracted from plant source by extraction processes like aqueous extraction and methanolic extraction. In this study microbes used are *E.coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* Gram-positive bacteria such as *Staphylococcus aureus* are mainly responsible for postoperative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (B.R. Ghalem and B. Mohamed, 2008). Gram-negative bacterium such as *Escherichia coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicemia (I. Bhattacharjee et al., 2005). Microbes have acquired specific type of resistance against antibiotic drugs which leads to the thought of developing more antimicrobial drugs. In this context to target various microbial infections, a great interest in the antibiotic property of medicinal plants has led to the production of plant-based antimicrobial drug molecules at the industrial scale. In India, from centuries several plants are used as medicinal plants. In which *Azadirachta indica* (Neem), is one of them and are highly used in rural and industrial area. In rural India, it is understood that Neem is highly effective in different type of disease e.g. skin disease in wound treatment. *Lantana sp.* and *Eucalyptus sp.* are also used to treat microbial diseases. Medicinal plant based industry take advantage from that plant and to invent new drugs for treatment of disease will be the new background for new researchers

MATERIAL AND METHOD

All the chemicals used for preparation of reagents were of AR, EP grade of Sigma, Merck or equivalent grade. The growth media used in microbiological work were readymade and supplied by Titan Biotech and Thomas Baker. Three species of bacteria, viz. *E. coli, Staphylococcus aureus, and Pseudomonas aruginosa* were used in the study and their stock cultures were obtained from culture that was procured from MTCC Unit of IMTECH, Chandigarh in the form of a slant. For plant extract, leaf and bark of *Azadirachta indica***,** *Lantana camara***,** *and Eucalyptus sp.* were procured from Bithoor area in Kanpur and tannery effluent polluted area of Banther in Unnao and Jajmau in Kanpur. All the plants were identified at Botany Dept., D.B.S. College Kanpur.

In order to extract phytochemicals from plant leaves and bark, methanolic and aqueous extraction methods were used. The bacteria cultures were grown in Brain Heart Infusion liquid medium at 37°C. After 6 hours of growth, each microorganism, at a concentration of 10^6cells/mL, was inoculated on the surface of agar plates. Subsequently, filter paper discs (6 mm in diameter) saturated either with extract or phytochemicals (50 µL) were placed on surface of each inoculated plate. To evaluate the efficiency of the methodology,

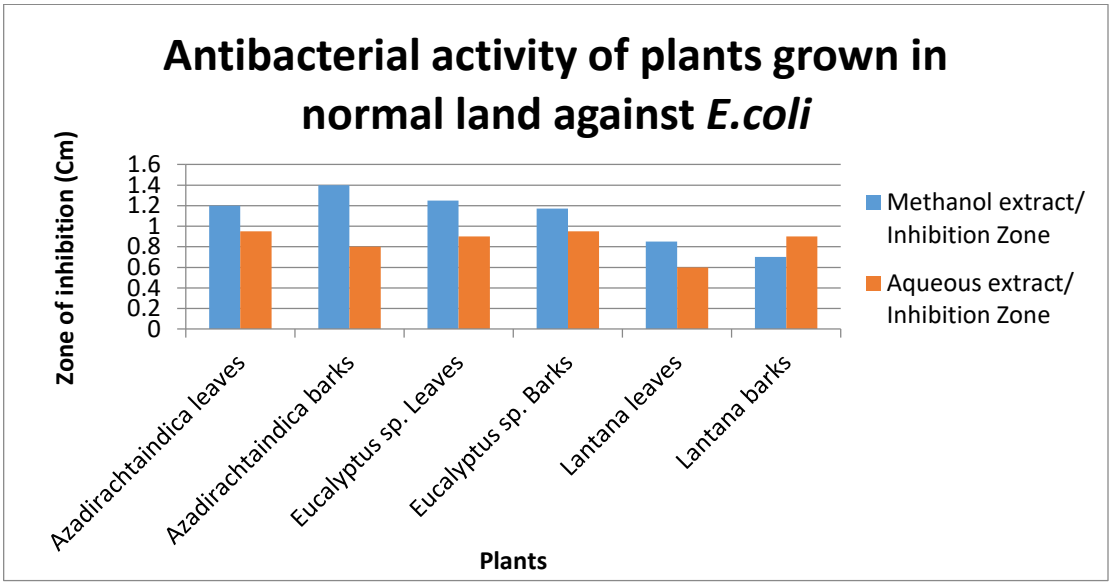
each extract was poured simultaneously in the wells made in new plates. The plates were incubated at 37 °C for 24 h. After this period, it was possible to observe inhibition zone. 2% DMSO and Tween 80 were used to dissolve the extracts in the culture media when necessary. The controls were the solvents used for each extract and the phytochemicals and they showed no inhibitions in preliminary studies. The extracts and the phytochemicals that showed antibacterial activity were later tested to determine the Minimal Inhibitory Concentration (MIC) for each bacterial sample. All the three bacterial samples [*E coli, Staphylococcus aureus, and Pseudomonas aruginosa*] were grown in nutrient broth for 6 hour. 100 µL of 10^6 cells/mL was inoculated in tubes with nutrient broth supplemented with different concentrations (10 – 500µL) of the extracts and phytochemicals, respectively. After 24 hours at 37 °C, the MIC of each sample was determined (Bauer 1966 ; Bayer et al., 1966), (Nascimento et al., 2000).

RESULTS

The antibacterial activity of the selected plant, *Azadirachta indica, Eucalyptus sp., Lantana camara* grown in two different conditions viz. normal land and tannery effluent polluted land were measured by the disc diffusion method against three bacterial species viz. *E.coli, Pseudomonas aruginosa, Staphylococcus aureus* by aqueous and methanolic extract and results were given in Table 1-6. The bacterial strains were gram positive bacteria and gram negative bacteria. The extracts of all selected plants investigated for antibacterial potential, all the plants extracts exhibited antibacterial activity against all the selected bacterial culture. Comparative study of antimicrobial activity for all three bacteria viz. *E. coli, Staphylococcus aureus, and Pseudomonas aeruginosa* of plants grown in unpolluted areas vs tannery polluted areas were also assessed.

Table 1: Antibacterial activity of plants grown in normal land for *E.coli*

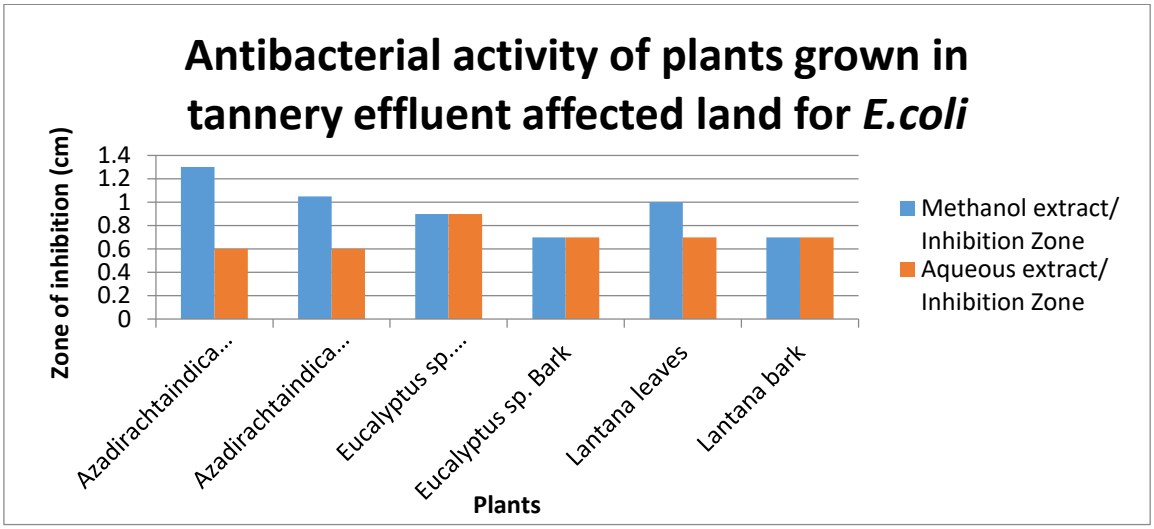
Plant	**Methanol extract/ Inhibition Zone**	**Aqueous extract/ Inhibition Zone**
Azadirachta indica leaves	1.2 cm.	0.95 cm.
Azadirachta indica barks	1.4 cm.	0.8 cm.
Eucalyptus sp. Leaves	1.25 cm.	0.9 cm.
Eucalyptus sp. Barks	1.17 cm.	0.95 cm.
Lantana leaves	0.85 cm.	0.6 cm.
Lantana barks	0.7 cm.	0.9 cm.



Graph 1: Antibacterial activity of plants grown in normal land for *E.coli*

Table 2: Antibacterial activity of plants grown in tannery effluent affected land for *E.coli*

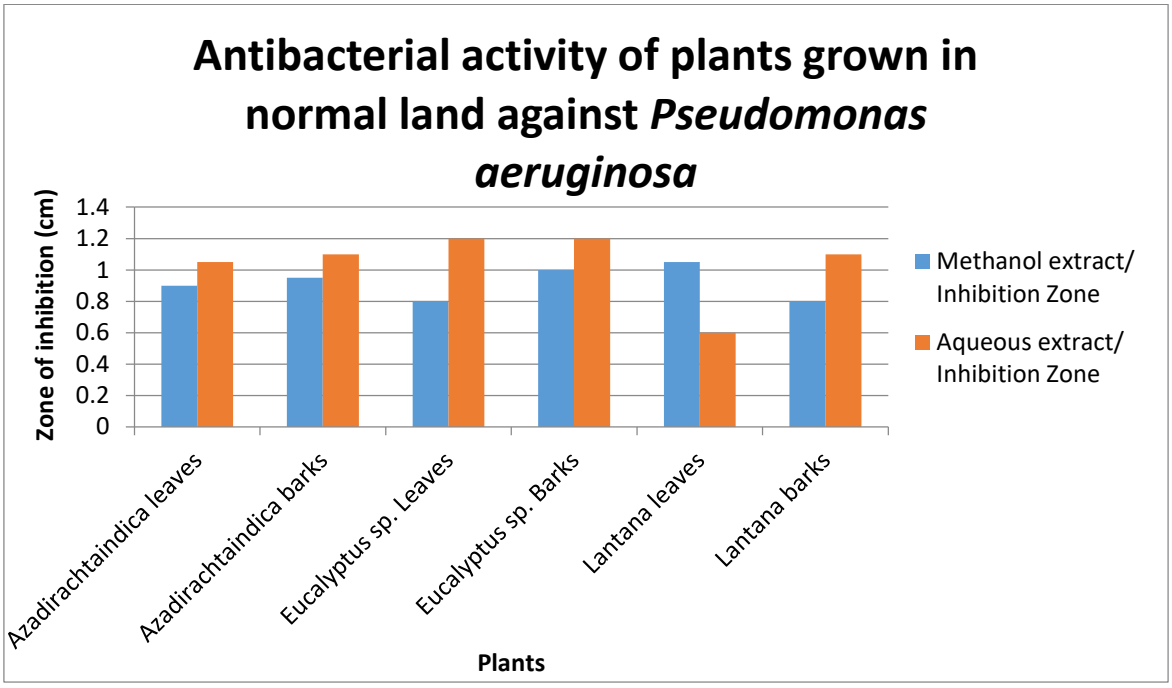
Plants	**Methanol extract/ Inhibition Zone**	**Aqueous extract/ Inhibition Zone**
Azadirachta indica leaves	1.3 cm.	0.6 cm.
Azadirachta indica bark	1.05 cm.	0.6 cm.
Eucalyptus sp. leaves	0.9 cm.	0.9 cm.
Eucalyptus sp. bark	0.7 cm.	0.7 cm.
Lantana leaves	1.0 cm.	0.7 cm.
Lantana bark	0.7 cm.	0.7 cm.



Graph 2: Antibacterial activity of plants grown in tannery effluent affected land against *E.coli*

Table 3: Antibacterial activity of plants grown in normal land for *Pseudomonas aeruginosa*

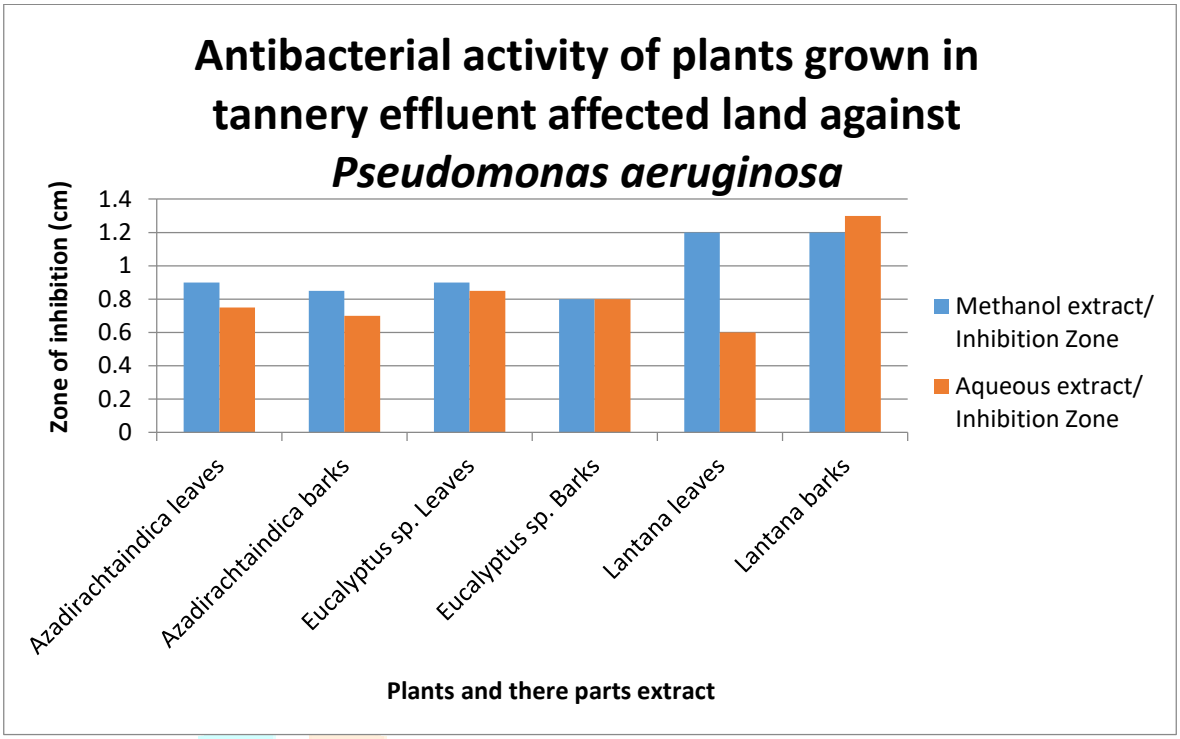
Plant	**Methanol extract/ Inhibition Zone**	**Aqueous extract/ Inhibition Zone**
Azadirachta indica leaves	0.9 cm.	1.05 cm.
Azadirachta indica barks	0.95 cm.	1.1 cm.
Eucalyptus sp. leaves	0.8 cm.	1.2 cm.
Eucalyptus sp. barks	1.0 cm.	1.2 cm.
Lantana leaves	1.05 cm.	0.6 cm.
Lantana barks	0.8 cm.	1.1 cm.



Graph 3: Antibacterial activity of plants grown in normal land against *Pseudomonas aeruginosa*

Table 4: Antibacterial activity of plants grown in tannery effluent affected land against *Pseudomonas aeruginosa*

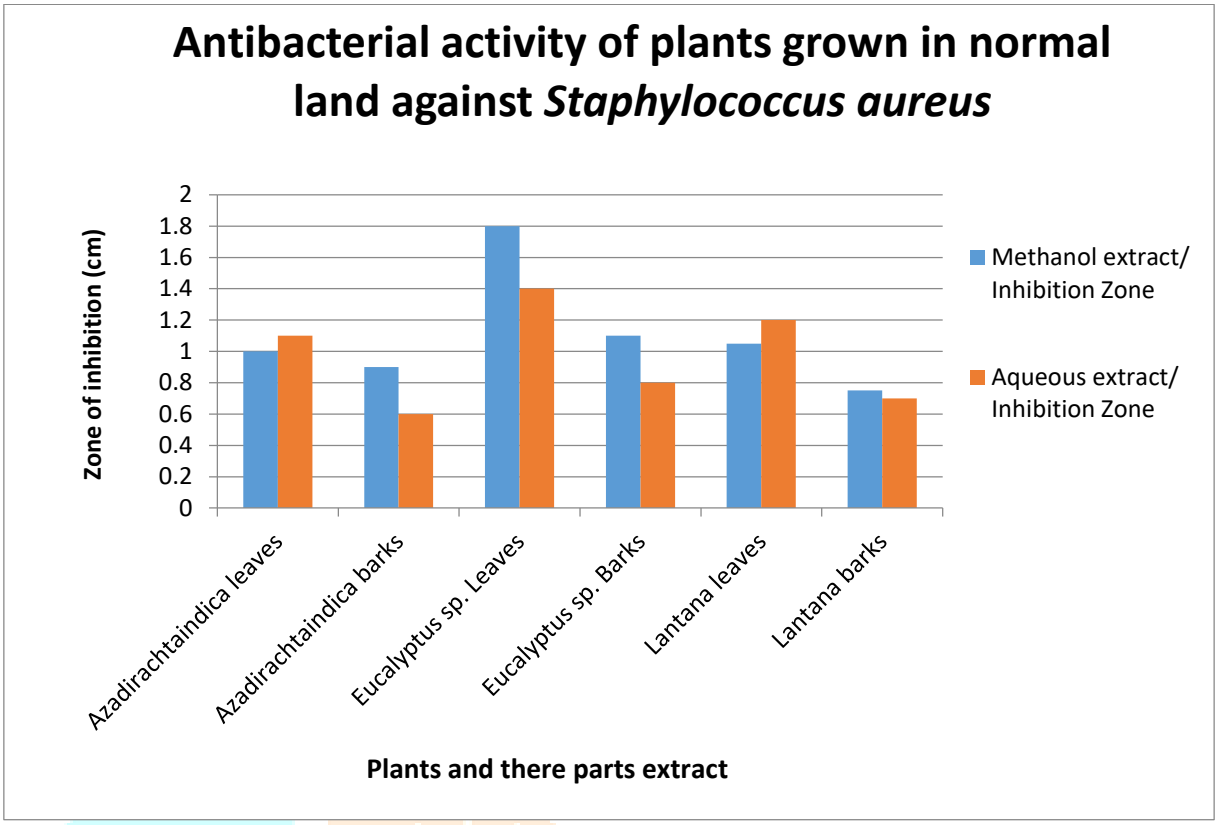
Plants	**Methanol extract/ Inhibition Zone**	**Aqueous extract/ Inhibition Zone**
Azadirachta indica leaves	0.9 cm.	0.75 cm.
Azadirachta indica barks	0.85 cm.	0.7 cm.
Eucalyptus sp. leaves	0.9 cm.	0.85 cm.
Eucalyptus sp. barks	0.8 cm.	0.8 cm.
Lantana leaves	1.2 cm.	0.6 cm.
Lantana barks	1.2 cm.	1.3 cm.



Graph 4: Antibacterial activity of plants grown in tannery effluent affected land against *Pseudomonas aeruginosa*

Table 5: Antibacterial activity of plants grown in normal land against *Staphylococcus aureus*

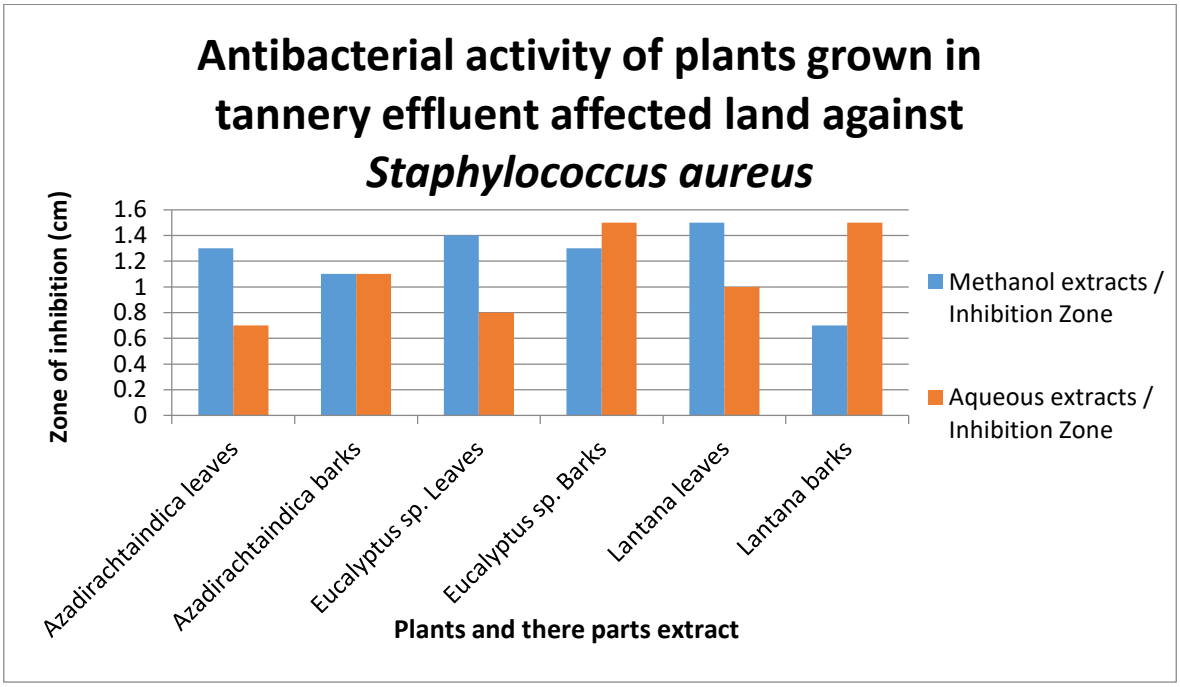
Plants	**Methanol extract/ Inhibition Zone**	**Aqueous extract/ Inhibition Zone**
Azadirachta indica leaves	1.0 cm.	1.1 cm.
Azadirachta indica barks	0.9 cm.	0.6 cm.
Eucalyptus sp. Leaves	1.8 cm.	1.4 cm
Eucalyptus sp. Barks	1.1 cm.	0.8 cm.
Lantana leaves	1.05 cm.	1.2 cm.
Lantana barks	0.75 cm.	0.7 cm.



Graph 5: Antibacterial activity of plants grown in normal land against *Staphylococcus aureus*

Table 6: Antibacterial activity of plants grown in tannery effluent affected land against *Staphylococcus aureus*

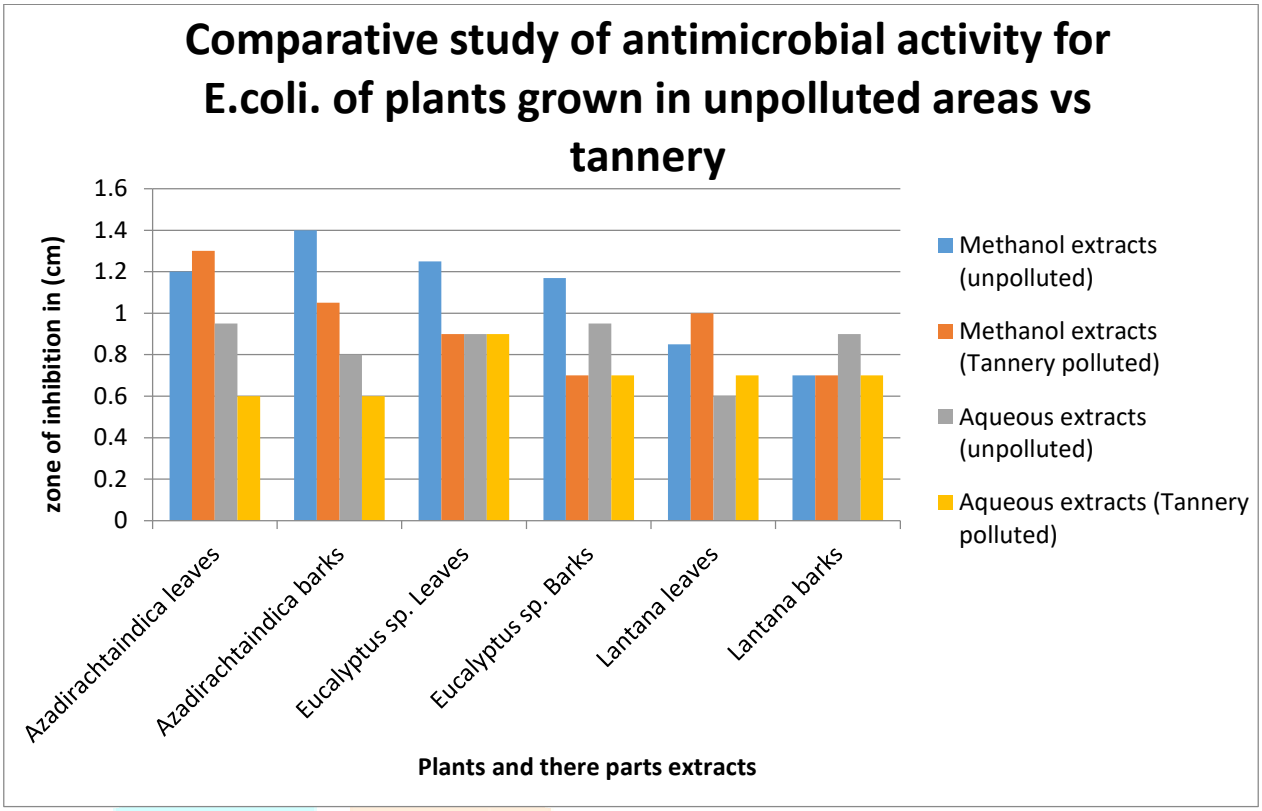
Plants	**Methanol extracts / Inhibition Zone**	**Aqueous extracts / Inhibition Zone**
Azadirachta indica leaves	1.3 cm.	0.7 cm.
Azadirachta indica barks	1.1cm.	1.1cm.
Eucalyptus sp. Leaves	1.4cm.	0.8cm
Eucalyptus sp. Barks	1.3cm.	1.5 cm.
Lantana leaves	1.5 cm.	1.0cm.
Lantana barks	0.7 cm.	1.5 cm.



Graph 6: Antibacterial activity of plants grown in tannery effluent affected land against *Staphylococcus aureus*

Table 7: Comparative study of antimicrobial activity for E.coli of plants grown in unpolluted areas vs tannery polluted areas

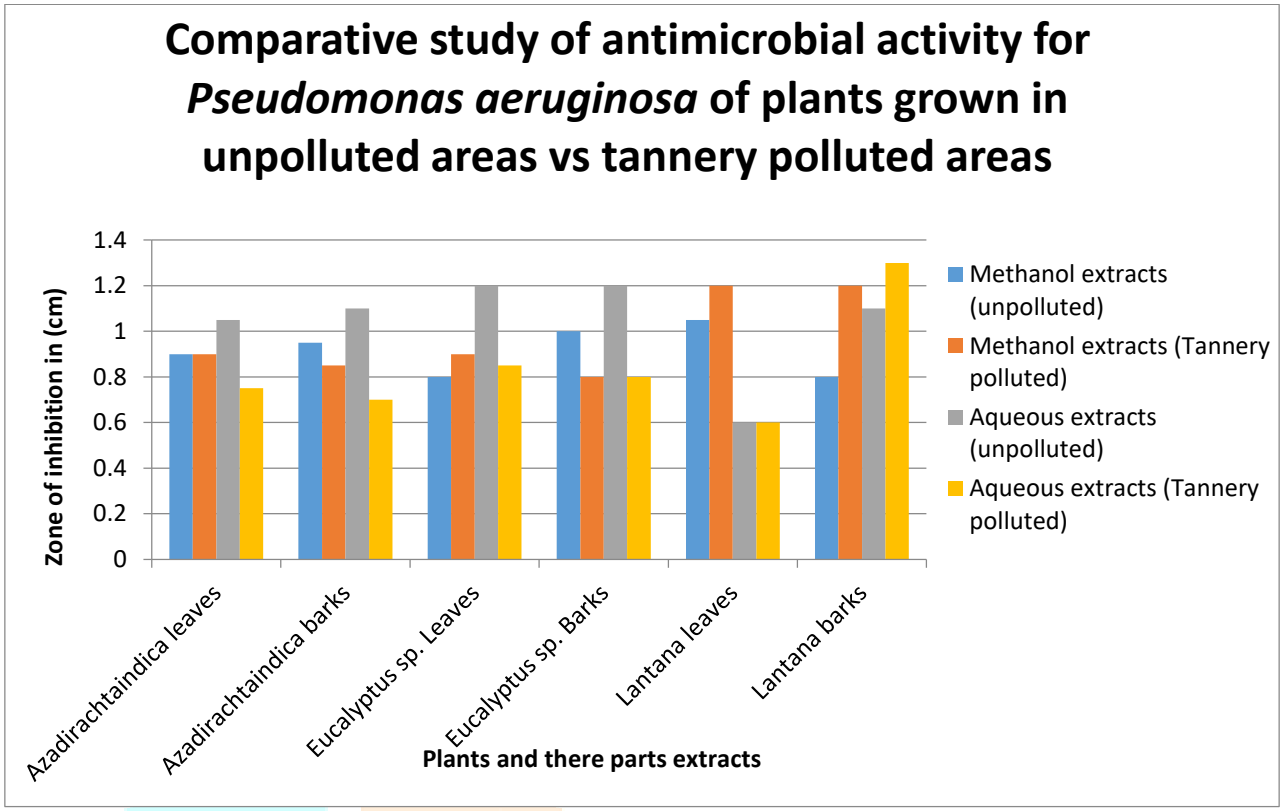
Plants	**Methanol extracts (unpolluted)**	**Methanol extracts (Tannery polluted)**	**Aqueous extracts (unpolluted)**	**Aqueous extracts (Tannery polluted)**
Azadirachta indica leaves	1.2 cm.	1.3 cm.	0.95 cm.	0.6 cm.
Azadirachta indica barks	1.4 cm.	1.05 cm.	0.8 cm.	0.6 cm.
Eucalyptus sp. Leaves	1.25 cm.	0.9 cm.	0.9 cm.	0.9 cm.
Eucalyptus sp. Barks	1.17 cm.	0.7 cm.	0.95 cm.	0.7 cm.
Lantana leaves	0.85 cm.	1.0 cm.	0.6 cm.	0.7 cm.
Lantana barks	0.7 cm.	0.7 cm.	0.9 cm.	0.7 cm.



Graph 7: Comparative study of antimicrobial activity for E.coli of plants grown in unpolluted areas vs tannery polluted areas

Table 8: Comparative study of antimicrobial activity for *Pseudomonas aeruginosa* of plants grown in unpolluted areas vs tannery polluted areas

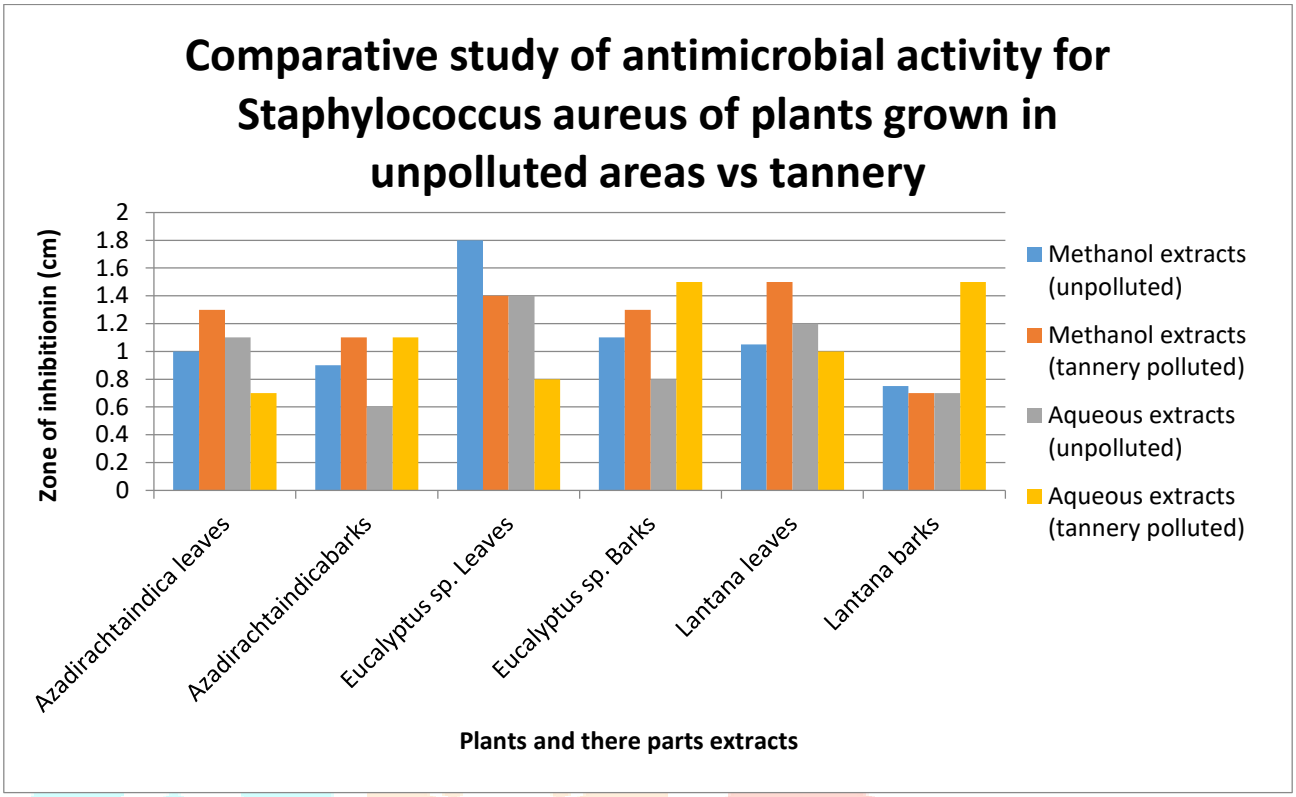
Plants	**Methanol extracts (unpolluted)**	**Methanol extracts (Tannery polluted)**	**Aqueous extracts (unpolluted)**	**Aqueous extracts (Tannery polluted)**
Azadirachta indica leaves	0.9 cm.	0.9 cm.	1.05 cm.	0.75 cm.
Azadirachta indica barks	0.95 cm.	0.85 cm.	1.1 cm.	0.7 cm.
Eucalyptus sp. Leaves	0.8 cm.	0.9 cm.	1.2 cm.	0.85 cm.
Eucalyptus sp. Barks	1.0 cm.	0.8 cm.	1.2 cm.	0.8 cm.
Lantana leaves	1.05 cm.	1.2 cm.	0.6 cm.	0.6 cm.
Lantana barks	0.8 cm.	1.2 cm.	1.1 cm.	1.3 cm.



Graph 8: Comparative study of antimicrobial activity for *Pseudomonas aeruginosa* of plants grown in unpolluted areas vs tannery polluted areas

Table 9: Comparative study of antimicrobial activity for *Staphylococcus aureus* of plants grown in unpolluted areas vs tannery polluted areas

Plant extracts	**Methanol extracts (unpolluted)**	**Methanol extracts (tannery polluted)**	**Aqueous extracts (unpolluted)**	**Aqueous extracts (tannery polluted)**
Azadirachta indica leaves	1.0 cm.	1.3 cm.	1.1 cm.	0.7 cm.
Azadirachta indica barks	0.9 cm.	1.1cm.	0.6 cm.	1.1cm.
Eucalyptus sp. leaves	1.8 cm.	1.4cm.	1.4 cm	0.8cm
Eucalyptus sp. barks	1.1 cm.	1.3cm.	0.8 cm.	1.5 cm.
Lantana leaves	1.05 cm.	1.5 cm.	1.2 cm.	1.0cm.
Lantana barks	0.75 cm.	0.7 cm.	0.7 cm.	1.5 cm.



Graph 9: Comparative study of antimicrobial activity for *Staphylococcus aureus* of plants grown in unpolluted areas vs tannery polluted areas

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