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Research Article

Comparative Study on Antimicrobial Activity and Microbial Load of *Alternanthera philoxeroides* (Mart.) Griseb Collected from Polluted and Unpolluted Site

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The present study analyses the antimicrobial activity and the microbial load of an edible plant *Alternanthera philoxeroides* (Mart.) Griseb growing in polluted and unpolluted site. The plants were collected and tested against various Gram positive, Gram negative bacteria and fungi. Antimicrobial activity was performed with acetone, aqueous, chloroform, ethanol and petroleum ether extracts of aerial parts *A. philoxeroides* collected from polluted and unpolluted site that showed significant antimicrobial activity against tested bacterial and fungal organisms. The extracts were compared with standards like Amoxicillin for antibacterial activity and Ketoconazole for antifungal activity. The extracts showed remarkable antimicrobial activity as measured from the zone of inhibition and results were comparable with that of standard drugs against the organisms tested. The microbial load is also enumerated in the cooked and cooked refrigerated samples from polluted and unpolluted site. In conclusion, plant extract of *A. philoxeroides* collected from polluted site showed less antimicrobial activity and higher antimicrobial activity in unpolluted site. The ethanol extract showed higher activity when compared to other extracts. The microbial load is higher in cooked refrigerated sample when compared to cooked sample.

Keywords: *Alternanthera philoxeroides*, antimicrobial activity and microbial load.

INTRODUCTION

Medicinal plants used as antimicrobial agents to avoid the development of multi-drug resistant bacteria; they were acting by different mechanisms. Now-a-days multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Davis 1994; Service 1995). Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. Leaf is one of the highest accumulated plant part of such compounds and people generally preferred it for therapeutic, purposes some of the active compounds inhibit the growth of disease causing microbes either singly or in combination (Hassawi and Kharma, 2006). Medicinal plants have been tested for biological, antimicrobial and hypoglycemic activity. They have also tested for antiulcerogenic, antihelminthic, hepatoprotective, analgesic, antipyretic, antileishmania and insecticidal activities (Doughari and Obidah, 2008). Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world (Nair and Chanda, 2004; Nair *et al*., 2000).

Alternanthera philoxeroides (Mart.) Griseb belonging to Amaranthaceae family is aquatic and semi-aquatic herbaceous plant (Steve and Anna 2010). This plant is useful in influenza, diarrhea, dysentery, stomach disorders etc. (Kumar *et al*., 2011; Niu 1986).

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Laboratory experiments give preventive measures against dengue (Jiang *et al.*, 2005), respiratory syncytial virus (Jiang *et al.*, 2007) and hemorrhagic fever virus (Yang *et al,* 1989). Gunasekera (1999) states that, *A. philoxeroides* is considered to be one of the worst aquatic weeds in the world. The aquatic form of the plant has the potential to become a serious threat to waterways, agriculture and the environment. Pollution is one of the serious problems faced globally today due to increased industrialization. Pollution has been described as a stress on plants since they respond to it in the same way as they respond to drought and any other environmental stress. Sometime the local people collect the plants from polluted site and sell in the market. It is very harmful to consumer health. Now-a-days, the human lifestyle is also changed extremely; most of the people cook the food and store it in the refrigerator. The microorganisms are growing rapidly in stored food in the fridge. The objective of this study was to investigate the antimicrobial activity and to quantify the microbial load of cooked sample and cooked sample stored in the refrigerator.

MATERIALS AND METHODS

Collection and authentication of plant materials

The *A. philoxeroides* specimens were collected from two different sites Pechiparai of Kanyakumari District (unpolluted site), and Cooum River Maduravoyal, Chennai Tamil Nadu (polluted site) and identified. Fresh plants were washed thoroughly three to four times with running tap water and sterile water, shade dried powdered and stored. Each sample of 10g were taken and soaked for 24h in 30ml of acetone, aqueous, chloroform, ethanol and petroleum ether separately. The extracts were filtered using Whatman filter paper No. 1, evaporated to dryness and re-dissolved in DMSO (Dimethyl Sulphoxide). The extracts were preserved in airtight container and kept at 4-5°C for further use.

Antibacterial Activity

The bacterial cultures used in the study were *Staphylococcus aureus* and *Enterococcus faecalis* (positive), *Klebsiella pneumoniae*, *Salmonella typhi* and *Escherichia coli* (negative). Antibacterial activity of solvent extracts was determined by well diffusion method on MHA medium. The bacterial culture to be tested was inoculated as lawn culture using sterile swab. Wells were made on to the agar plate using sterile cork borer (6mm diameter). The extracts were applied to different wells in serially increasing volumes 30µg, 40µg and 50µg. DMSO (Dimethyl Sulphoxide) served as negative control and Amoxicillin (10µg) used as the reference. The plates were labelled, covered and incubated at 37°C for 24h.

Antifungal Activity

The fungal cultures used in the study were *Aspergillus niger*, *Candida albicans*, *Epidermophyton floccosum*, *Microsporum gypseum* and *Penicillium chrysogenum*. The fungal mycelial suspension was spread on PDA plates and 6 mm diameter wells were made with cork borer. The extracts were applied to different wells in serially increasing volumes of 30µg, 40µg and 50µg. DMSO served as negative control whereas Ketoconazole (10µg) used as the reference. The plates were labelled, covered and incubated at 28°C for 3-6 days. The activity of the extracts was determined by measuring the diameter of zone of inhibition.

Enumeration of Microbial Load in Cooked Sample and Cooked Refrigerated Sample of polluted and unpolluted *A. philoxeroides*

The polluted and unpolluted pre-weighted sample *A. philoxeroides* washed thrice in 100ml of sterile water and cooked for 5-10 minutes at 80-90^{o}C separately. After cooking, the samples were removed and the water serially diluted and plated on Nutrient Agar (NA) plates. Triplicate plates of appropriate dilutions were prepared. The NA plates were incubated at 37°C for 24h. The microbial colonies were counted and tabulated. The cooked sample along with the water refrigerated for 8h at 8°C, serially diluted and plated on NA plates. The NA plates were incubated at 37°C for 24h. The microbial colonies were counted and tabulated.

RESULTS AND DISCUSSION

The acetone, aqueous, chloroform, ethanol, and petroleum ether extracts of *A. philoxeroides* were tested for growth inhibiting activity against five bacterial strains and five fungal strains in three varying concentrations. The results (Tables 1, 2, 3, 4 and 5) show that plants from the polluted site possess very poor antibacterial and antifungal activity. In polluted site, the ethanol extracts showed moderate microbial activity against the organisms tested. The ethanol extract of the plant was found to have more microbial activity than the rest of the extracts. The zone of inhibition was compared to that of the standard.

Table1 Antimicrobial Activity of the Acetone Extract of *Alternanthera philoxeroides*

Test organisms	Zone of inhibition in mm						
	Amoxicillin (10 µg/mL) Ketoconazole (10 µg/mL)	30 µg		40 µg		50 µg	
		P	UP	P	UP	P	UP
Staphylococcus aureus	27	8	13	9	15	11	15
Enterococcus faecalis	25	7	11	8	13	9	15
Klebsiella pneumoniae	23	-	11	9	13	10	14
Escherichia coli	25	-	10	9	12	11	14
Salmonella typhi	17	-	9	7	11	9	13
Aspergillus niger	21	-	9	-	10	9	11
Candida albicans	19	-	-	-	9	8	11
Epidermophyton floccosum	17	-	-	-	9	9	10
Microsporum gypseum	18	-	7	-	8	-	9
Penicillium chrysogenum	15	-	8	-	9	9	10

Table 2 Antimicrobial Activity of the Aqueous Extract of *Alternanthera philoxeroides*

Test organisms	Zone of inhibition in mm						
	Amoxicillin (10 µg/mL) Ketoconazole (10 µg/mL)	30 µg		40 µg		50 µg	
		P	UP	P	UP	P	UP
Staphylococcus aureus	27	-	11	8	13	9	15
Enterococcus faecalis	25	-	-	7	11	8	12
Klebsiella pneumoniae	23	-	11	6	13	7	15
Escherichia coli	25	-	9	7	11	8	13
Salmonella typhi	17	7	7	8	9	9	10
Aspergillus niger	21	8	-	9	9	10	11
Candida albicans	19	7	-	-	-	7	9
Epidermophyton floccosum	17	-	-	-	-	7	9
Microsporum gypseum	18	-	7	-	9	7	11
Penicillium chrysogenum	15	6	-	-	11	9	13

Table 3 Antimicrobial Activity of the Chloroform Extract of *Alternanthera philoxeroides*

Test organisms	Zone of inhibition in mm						
	Amoxicillin (10 µg/mL) Ketoconazole (10 µg/mL)	30 µg		40 µg		50 µg	
		P	UP	P	UP	P	UP
Staphylococcus aureus	27	-	11	-	13	8	16
Enterococcus faecalis	25	-	10	-	13	7	15
Klebsiella pneumoniae	23	-	-	-	12	8	13
Escherichia coli	25	-	-	-	11	7	14
Salmonella typhi	17		-	-	12	-	13
Aspergillus niger	21	-	-	-	9	-	12
Candida albicans	19	-	-	-	-	7	11
Epidermophyton floccosum	17	-	-	-	-	-	7
Microsporum gypseum	18	-	-	-	-	-	9
Penicillium chrysogenum	15	-	-	-	-	-	9

Table 4 Antimicrobial Activity of the Ethanol Extract of *Alternanthera philoxeroides*

Test organisms	Zone of inhibition in mm						
	Amoxicillin (10 µg/mL) Ketoconazole (10 µg/mL)	30 µg		40 µg		50 µg	
		P	UP	P	UP	P	UP
Staphylococcus aureus	27	11	17	11	19	13	21
Enterococcus faecalis	25	8	11	9	13	9	15
Klebsiella pneumoniae	23	9	9	11	11	12	14
Escherichia coli	25	7	11	9	13	11	15
Salmonella typhi	17	8	-	9	9	10	11
Aspergillus niger	21	9	9	10	10	11	13
Candida albicans	19	7	8	9	9	10	12
Epidermophyton floccosum	17	-	-	8	7	9	9
Microsporum gypseum	18	9	7	10	8	11	9
Penicillium chrysogenum	15	-	8	11	9	13	11

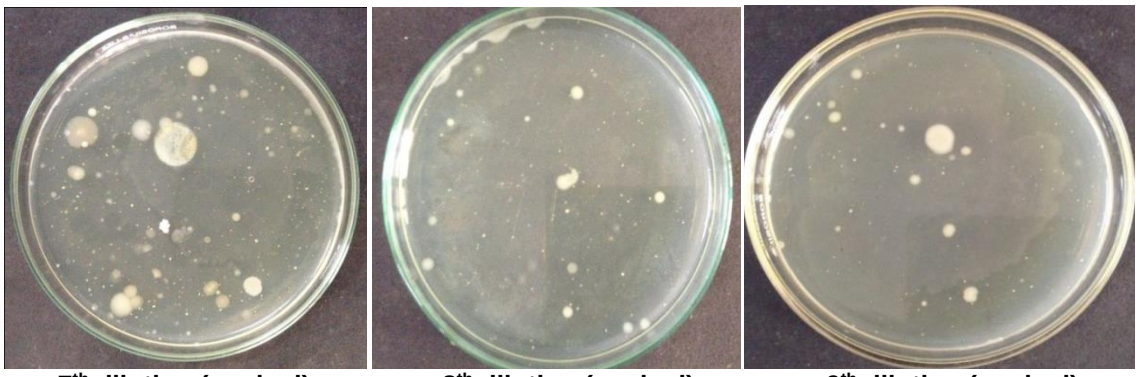
Table: 5 Antimicrobial Activity of the Petroleum Ether Extract of *Alternanthera philoxeroides*

Test organisms	Zone of inhibition in mm						
	Amoxicillin (10 µg/mL) Ketoconazole (10 µg/mL)	30 µg		40 µg		50 µg	
		P	UP	P	UP	P	UP
Staphylococcus aureus	27	-	11	7	13	9	17
Enterococcus faecalis	25	-	9	-	11	8	13
Klebsiella pneumoniae	23	-	-	7	9	9	11
Escherichia coli	25	-	9	6	11	7	13
Salmonella typhi	17	-	-	7	9	9	11
Aspergillus niger	21	-	-	8	9	9	12
Candida albicans	19	-	-	-	9	-	11
Epidermophyton floccosum	17	-	-	-	-	9	9
Microsporum gypseum	18	-	-	-	-	-	9
Penicillium chrysogenum	15	-	-	-	9	-	11

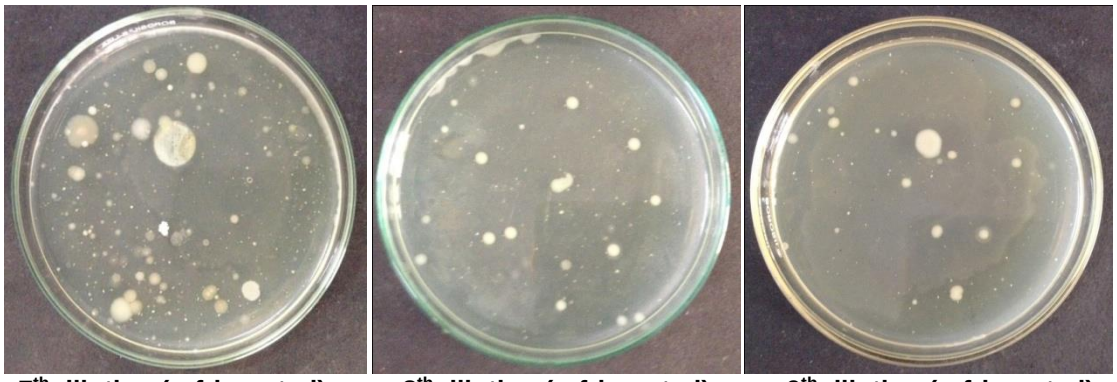
Note: UP - Unpolluted and P-polluted site plant of *A. philoxeroides*.

Table 6: Analysis of Microbial Load in Cooked and Cooked Sample Stored in Refrigerator 8h(Unpolluted Site)

Selected Dilutions for microbial Load	Cooked Sample	Cooked Sample Stored in Refrigerator (8h)
7^{th} Dilution	98×10^7	152×10^7
8^{th} Dilution	72×10^8	123×10^8
9^{th} Dilution	68×10^9	98×10^9



7^{th} dilution (cooked) **8^{th} dilution (cooked)** **9^{th} dilution (cooked)**



7^{th} dilution (refrigerated) **8^{th} dilution (refrigerated)** **9^{th} dilution (refrigerated)**

Figure 1: Microbial Load in Cooked and Cooked Sample Stored in Refrigerator (Unpolluted Site)

Table 7: Analysis of Microbial Load in Cooked and Cooked Sample Stored in Refrigerator (Polluted Site)

Selected Dilutions for Microbial Count	Cooked Sample	Cooked Sample Stored in Refrigerator (8h)
7^{th} Dilution	Uncountable	Uncountable
8^{th} Dilution	172x 10^8	265 x 10^8
9^{th} Dilution	127 x 10^9	198 x 10^9

7^{th} Dilution (Cooked) **8^{th} Dilution (Cooked)** **9^{th} Dilution (Cooked)**

7^{th} Dilution (Refrigerated) **8^{th} Dilution (Refrigerated)** **(9^{th} Dilution (Refrigerated)**

Figure 2: Microbial Load in Cooked and Cooked Sample Stored in Refrigerator (Polluted site)

The chloroform extract of the plant was found to inhibit *Microsporum gypseum* and *Penicillium chrysogenum* at the higher concentration (50µg). Pamila and Karpagam (2017a) reported that ethanol extracts showed the presence of fourteen major phytoconstituents in unpolluted site and nine major phytoconstituents in polluted site plant *A. philoxeroides.* Sowjanya Pulipati *et al.* (1975) reported the results from MIC indicated that *S. aureus* and *E.coli* were the most sensitive bacteria to *A. philoxeroides* leaf extract, inhibited at lowest concentration of 12.5µg/ml.

The presence of carbohydrates, amino acids, proteins, cardiac glycosides, steroids, alkaloids, flavonoids, total phenolics and tannin contents were reported in *A. philoxeroides* (Fang *et al*., 2006). The GC-MS analysis of the ethanolic extract of plants of *A. philoxeroides* and *A. bettzickiana* aerial parts showed the presence of many bioactive compounds. Five compounds are commonly present in both plants, they are n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z), Ar-tumerone, Bicyclo [3.1.1] heptane, 2,6,6-trimethyl, and Phenol, 5-(1,5-dimethyl-4-hexeny (Pamila and Karpagam, 2017b).

Microbial control is very important in food industry to prevent food poisoning and other health hazards. The results of the microbial load of cooked and cooked refrigerated sample *A. philoxeroides were* presented in Tables 6 and 7. The analysis of microbial load is 98 x10^7cfu/g, 72x10^8cfu/g, 68 x10^9cfu/g in cooked sample from unpolluted site and 152x10^7cfu/g, 123x10^8cfu/g, 98x10^9cfu/g in cooked refrigerated sample from unpolluted site. The results of the microbial load in cooked and cooked refrigerated samples from polluted site indicated a higher microbial growth. In polluted site fresh plant, the microbial load is uncountable in first wash, the microbial load is 187x10^9cfu/g in second wash; and the microbial load is 69x10^9cfu/g in third wash. In unpolluted site fresh plant, the microbial load is 57x10^9cfu/g in first wash, 34x10^9cfu/g in second wash and 13x10^9cfu/g in third wash (Pamila and Karpagam, 2017a). The leaves and stem are the sources of the natural dimeric alkaloids vinblastine and vincristine that are essential parts of most anti-cancer chemotherapies (Heijden *et al*., 2004).

CONCLUSION

The ethanol extract of the plant was found to have more microbial activity than the other extracts. In unpolluted site, the microbial populations in plant aerial parts were higher when compared to the polluted site. The result of the microbial load is higher in cooked refrigerated sample when compared to cooked sample. The microbial load increased in polluted site indicative of the fact that these plants had good resistance and tolerance to pollution. The findings from this study reiterate the need for constant quality assessment of herbal materials in the market in order to ensure that medicinal plant materials and products are suitable for human consumption. Medicinal plants sold in markets should be placed in clean sterile baskets or suitable hygienic packs.

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