Effect of environmental pollution on the quality of an edible plant Alternanthera philoxeroides (Mart.) Griseb

U. Arul Pamila1* and S. Karpagam2

1,2 Department of Botany, Queen Mary's College (A), Chennai, India.

The present study is the comparative analysis of phytochemical constituents and microbial load of an edible plant Alternanthera philoxeroides (Mart.) Griseb collected from unpolluted and polluted site. Preliminary phytochemical analysis was performed with acetone, aqueous, chloroform, ethanol and petroleum ether extracts (unpolluted and polluted site) of A philoxeroides that showed the presence of alkaloids, carbohydrates, saponins, phenols, flavonoids, aminoacids, diterpenes, tannin, terpenoids, protein, steroid, oxalate, coumarin and quinones. The ethanol extract showed higher number of phytochemical constituents when compared to the other extract of unpolluted site. The microbial load is also enumerated in the unpolluted and polluted site. In conclusion, phytochemical analysis revealed the presence of many phytoconstituents in ethanol extract and the microbial load is less in the unpolluted site when compared to the polluted site.

Keywords: Alternanthera philoxeroides, phytochemical constituents, microbial load.

INTRODUCTION

Medicinal plants have been a basic source of antibiotics against a variety of diseases over the years. Herbal plants are known to be the excellent stimulators of immune system due to antioxidant properties of phytochemical constituent. Herbal plants have been recently popularized in modern medicine, since many therapeutically important compounds are derived from them (Ghosh et al., 2006). Herbal plant based drugs have been in use against various infections (Ahmad et al., 2009). In the past, medicinal plants was the first line of treatment known to man and traditional medicinal practice remain an important part of the primary healthcare delivery system in most of the developing world (Akerele., 1998). Phytochemicals are naturally occurring in the medicinal plants in all the parts namely, leaves, stem and roots that have defense mechanism and protect from various diseases. Phytochemicals are of primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoids, alkaloids and phenolic compounds (Wadood et al, 2013; Krishnaiah et al., 2007). Plants accumulate various secondary metabolites including alkaloids, glycosides, polyphenols etc. They are effective in the treatment of infectious diseases with minimal side effects that are often associated with synthetic antimicrobials (Iwu, et. al., 1999). A good number of drugs that are being used today are isolated from plant sources and some from animals or minerals. Alternanthera Forsskaal species are invasive aquatic weeds posing a strong threat to agro-biodiversity in several countries in the world. Weeds are a continuous and ubiquitous threat to agricultural productivity (Riaz et al., 2009).

*Corresponding author: U. Arul Pamila, Department of Botany, Queen Mary's College (A), Chennai, India. pamilastatin2004@gmail.com
Alternanthera philoxeroides (Mart.) Griseb (Amaranthaceae) is commonly known as alligator weed and joyweeds (Fig. 1) that can be found in many parts of the world, infesting rivers, lakes, ponds and irrigation canals, as well as many terrestrial habitats. It is an emergent stoloniferous perennial herb. The leaves are dark green, elliptic, glabrous and opposite, 3.5-7.1 cm long and 0.5-2 cm wide. Mature aquatic plants have hollow stems up to 10 m long that form thick interwoven mats throughout the water body and emerge up to 20 cm out of the water when the plant flowers. Flowers are white, inflorescences terminal and axillary, 1.4-1.7 cm in diameter, on a short stalk. In the native range the species is known to set seed. In much of the invasive range seed production is not observed (Bassett, 2006).

Habitats: Although more suited to aquatic and riparian habitats, where the species forms dense mats in shallow slow-moving water bodies, it is also a vigorous colonizer of terrestrial habitats where the extensive (up to 2 m) deep rhizome system can sustain the population throughout extended dry periods. Often, it grows at the interface between the aquatic and terrestrial environment. Spread is predominantly vegetative, from axillary buds at each node in the warm summer months. (Julien and Bourne, 1988) list a number of habitats which will sustain populations of the species, including, but not exclusive too, freshwater habitats, coastal areas, managed terrestrial habitats including cultivated agricultural land, disturbed areas and urban habitats. In addition, natural and semi-natural habitats are prone to invasion, including forests, riverbanks and wetlands (Clements et al., 2014). It is an emergent, semi-aquatic plant which originated in South America, introduced range includes New Zealand, Australia, Asia, and more recently parts of Europe (Julien, 1995); A. philoxeroides is found throughout India, including Assam, Bihar, West Bengal, Tripura, Manipur, Andhra Pradesh, Karnataka, Maharashtra, Delhi and the state of Punjab (Pramod et al., 2008).

**A philoxeroides** plays a vital role in human health care. All parts of plants are used in traditional systems of medicine for the treatment of numerous human ailments. They were reported to treat bacterial, fungal and viral diseases. Young tender shoots and leaves of A.philoxeroides are eaten as a vegetable in Southeast Asia (Scher Federal., 2009). The plant is used as leafy vegetable. It contains an immense variety of bioactive compounds such as antimicrobials, antioxidants, phytochemicals, essential fatty acids and dietary fibres (Gupta and Prakash., 2008). Due to its dietary importance, many scientific studies have been carried out on the nutritive values of herbal plant of A. philoxeroides (Gayathri et al., 2006). Therefore, the present work is aimed to compare and explore the microbial load and phytochemical analysis of Aphiloxeroides collected from unpolluted and polluted sites Pechiparai of Kanyakumari District, Tamil Nadu and Cooum River, Maduravoyal (Fig. 2).

**MATERIALS AND METHODS**

**Collection and authentication of plant materials**

The A. philoxeroides specimens were collected from two different sites Pechiparai of Kanyakumari District, Tamil Nadu and Cooum River Maduravoyal, Chennai. The collected plants were identified in the Department of Botany, Queen Mary's College and confirmed by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC) Chennai.

**Plant material**

Fresh plants were washed thoroughly three to four times with running tap water then finally with sterile water followed by shade drying at room temperature for 20-30
days and powdered by using an electric blender and stored in airtight container.

**Preparation of extract**

Each sample of 10g were taken and soaked for 24h in 30ml of aqueous, acetone, 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.

**Enumeration of microbial load from fresh plants**

Freshly collected plant twig of 10 cm was washed in 100ml of sterile water; the twig was placed on sterile blotting paper to remove excess water and again washed in 100ml of sterile water and repeated again for third wash. The three sterile waters were serially diluted and plated on NA plates separately.

The pour plate method was used to cultivate serially diluted portions of the medicinal plant samples under investigation. Enumeration was carried out on nutrient agar. Triplicate plates of appropriate dilutions were prepared. The NA plates were incubated at 37°C for 24h for bacterial growth. The developed microbial colonies were counted and tabulated.

**Enumeration of microbial load from powdered plant samples**

The plant sample (0.1g) was added to 20ml of lukewarm NA media, mixed thoroughly and poured in Petriplates, incubated it for 24h and observed for bacterial colonies. The developed microbial colonies were counted.

The plant sample (0.1g) was suspended in 1ml sterile water shaken well and serially diluted and 0.1 ml was plated on NA plates and incubated. After 24h, the developed microbial colonies were counted and tabulated.

**Phytochemical Analysis**

The plants have primary and secondary metabolites which can be used for medicinal and other uses. There is a need to analyze the plants for such phytochemical screening. Phytochemical screening was carried out by using the standard protocols as described by Harborne (1973). The alkaloids are determined by Wagner’s Test (Tiwari et al., 2011); carbohydrates by Benedict’s Test; saponin by Foam Test phenol by Ferric Chloride Test; flavonoids by Lead Acetate Test; diterpenes by Copper Acetate Test; aminoacids by Ninhydrin Test and Tannins by Ferric Chloride Test; terpenoids by Salkowski’s Test, proteins by Biuret Test and Tannins by Ferric Chloride Test; terpenoids by Salkowski’s Test, proteins by Biuret Test (Khanam et al., 2014) and oxalate by Ethanoic acid glacial (Ugochukwu et al., 2013). Further detection of steroids was carried out by Harborne (1973); detection of coumarin was done by Mace method (Mace, 1963) and quinone by conc. H₂SO₄. Xanthoproteins by conc. HNO₃ and NH₃ Test, proteins by Biuret Test, carboxylic acid by effervescence test (Kumar et al., 2013) cardiac glycosides by Kellerkillani synthesis (Misra et al., 2011) anthocyanin by HCl and NH₃, leucoanthocyanin by isoamyl alcohol (Ashvin Godghate et al., 2012); and glycosides by Modified Borntrager’s Test (Kokate et al., 2006).
RESULTS AND DISCUSSION

The phytochemical analysis was carried out from the dried (polluted site and unpolluted site) plant powder using aqueous, acetone, chloroform, ethanol, and petroleum ether, which showed the presence of many bioactive compounds in the plant in Table 1. Twenty different phytochemical tests were carried out for the five different extracts. Ethanol extracts showed the presence of fourteen major phytoconstituents in unpolluted site plant and nine major phytoconstituents in polluted site plant. The preliminary phytochemical analysis showed the presence of alkaloids, carbohydrates, saponins, phenols, flavonoids, aminoacids, diterpenes, tannin, terpenoids, protein, steroid, oxalate, anthocyanin, leucaanthocyanin, Xanthoprotein, coumarin and quinone in the ethanol extract of Alternanthera philoxeroides (Mart.) Griseb. The presence of these metabolites suggests great potential for the plant as a source of useful phytomedicines (Kunle et al., 2003). Antibiotic activities are common for alkaloids some are even used as antiseptics in medicines (Cordell 1983).

The preliminary phytochemical analysis showed primary and secondary metabolites such as alkaloids, carbohydrates, saponins, phenols, flavonoids, diterpenes, tannins, terpenoids, steroid, oxalate, anthocyanin, leucaanthocyanin, Xanthoprotein, coumarin and glycosides in the ethanol extract of A. bettzickiana (Pamila and Karpagam, 2017). The presence of carbohydrates, aminoacids, proteins, cardiac glycosides, alkaloids, steroids, flavonoids, total phenolics and tannin contents were reported in A. philoxeroides (Fang et al., 2006). The medicinal value of plants depends on the presence of phytoconstituents. The chloroform extract showed the presence in minimal amount of phytoconstituents. The results showed the presence of some secondary metabolites which proves that the plant is of great medicinal values.

The results of the microbial load of fresh plant A. philoxeroides collected from different location namely unpolluted and polluted site are presented in Table 2. In the polluted site plant, the microbial load is uncountable in first wash; the microbial load is 187x10^9 cfu/g in second wash; and the microbial load is 69x10^9 cfu/g in third wash. In unpolluted site plant, the microbial load is 57x10^6 cfu/g in first wash, 34x10^9 cfu/g in second wash, and 13x10^9 cfu/g in third wash.

### Table 1: Phytochemical content of A. philoxeroides collected from unpolluted and polluted site.

<table>
<thead>
<tr>
<th>Name of the test</th>
<th>Ethanol</th>
<th>Chloroform</th>
<th>Acetone</th>
<th>Petroleum ether</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>UP</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Aminoacids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diterpenes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Protein</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oxalate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carboxylic acid</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xanthoprotein</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coumarin</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: "+" indicates present and "-" indicates absent. Anthocyanin and Leucoanthocyanin absent; UP: Unpolluted site plant and P: polluted site plant.

### Table 2: Analysis of microbial load of unpolluted and polluted site collected from fresh plant A. philoxeroides

<table>
<thead>
<tr>
<th>Washing a Twig of Plant</th>
<th>Unpolluted site (Fresh plant twig)</th>
<th>Polluted site (Fresh plant twig)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I wash</td>
<td>57 x 10^9</td>
<td>Uncountable</td>
</tr>
<tr>
<td>II wash</td>
<td>34 x 10^9</td>
<td>187 x 10^9</td>
</tr>
<tr>
<td>III wash</td>
<td>13 x 10^9</td>
<td>69 x 10^9</td>
</tr>
</tbody>
</table>
A. Fresh Plant Twig Polluted (*A. philoxeroides*)

(a) I wash, (b) II wash, (c) III wash

B. Fresh Plant Twig Unpolluted (*A. philoxeroides*)

(d) I wash, (e) II wash, (f) III wash

**Figure 1:** Photographs of Microbial Load of Fresh *A. philoxeroides* from polluted and Unpolluted Site

A. (a) I wash, (b) II wash, (c) III wash; B (e) I wash, (f) II wash, (f) III wash

C. Polluted Dry Plant Powder (*A. philoxeroides*)

(g) 7th dilution, (h) 8th dilution, (i) 9th dilution

D. Unpolluted Dry Plant Powder (*A. philoxeroides*)

(j) 7th dilution, (k) 8th dilution, (l) 9th dilution

**Figure 2:** Photographs of Microbial Load of Dry Plant Powder *A. philoxeroides* from polluted and Unpolluted Site

C. (g) 7th dilution, (h) 8th dilution, (i) 9th dilution; D. (j) 7th dilution, (k) 8th dilution, (l) 9th dilution
Table 3: Analysis of microbial load of unpolluted and polluted site dry plant powder of A. philoxeroides

<table>
<thead>
<tr>
<th>Plant dry powder (0.1g powder diluted 1ml of sterile water)</th>
<th>Unpolluted site</th>
<th>Polluted site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution 7</td>
<td>15 x 10⁷</td>
<td>36 x 10⁷</td>
</tr>
<tr>
<td>Dilution 8</td>
<td>10 x 10⁸</td>
<td>29 x 10⁸</td>
</tr>
<tr>
<td>Dilution 9</td>
<td>4 x 10⁹</td>
<td>17 x 10⁹</td>
</tr>
</tbody>
</table>

The results of the microbial load of dry powdered sample A. philoxeroides are presented in Table 3. The result of the microbial load of the polluted site dry plant powder sample counted 42 colonies and unpolluted site counted 21 colonies. The result of the microbial load of polluted site dry plant powder developed colonies 36 x 10⁷ cfu/g, 29 x 10⁸, and 17 x 10⁹, unpolluted site dry plant powder developed colonies 15 x 10⁷ cfu/g, 10 x 10⁶, and 4 x 10⁹ cfu/g. Contamination by microorganisms is dispensing hygiene is not good, contamination may be even worse (Obuekwe et al., 1998). Inadvertent contamination by microbial or chemical agents during processing could also caused deterioration, thereby compromising safety and quality, and rendering the medicinal plant material less effective and possibly affect the consumer (W.H.O., 2003). Thus, the result of the microbial load is higher amount in the polluted site when compared to the unpolluted site and also the microbial count is more in the fresh plant when compared to the dry plant powder.

CONCLUSION

These simple reliable standards will be useful to use herbs as a home remedy. Also, the manufacturers can utilize them for identification and selection of the raw material for drug production. The microbial load findings from this study emphasized the need for constant quality assessment of herbal drugs on sale in order to ensure the production of therapeutic products suitable for human consumption.

REFERENCES

Gayathri BM., Balasuriya K., De, G.S.P., Gunawardena, Rajapakse, R.P.V.J.
Effect of environmental pollution on the quality of an edible plant Alternanthera philoxeroides (Mart.) Griseb.


Accepted 25 April, 2017.


Copyright: © 2017 Pamila and Karpagam. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are cited.