

Phytochemical, Antimicrobial and Antioxidant properties of an invasive weed - *Chromolaena odorata* (L.) King & Robinson

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Abstract

Chromolaena odorata (L.) King & Rob. native to Tropical America belongs to Asteraceae family, is an invasive weed to Indian context. It spreads all across and started engulfing the forest fringes in Andhra Pradesh and other several states in India. With a view to positive utilization of the invasive weeds, the present work was initiated to study the phytochemical screening, antibacterial and antioxidant, activities of ethyl acetate, acetone, methanol and aqueous extracts of *Chromolaena odorata* leaves. Phytochemicals like Saponins, Alkaloids, Flavonoids, Steroids and Tannins were present in the ethyl acetate and aqueous extracts where as acetone and methanol extracts were positive for Flavonoids and Alkaloids. The ethyl acetate and aqueous extracts showed relatively more antibacterial activity than positive control streptomycin by exhibiting larger zone of diameter against *Proteus mirabilis* MTCC-425, *Bacillus megaterium* MTCC-428 and *Bacillus subtilis* MTCC-441. Ethyl acetate (58.34%) and aqueous (56.8%) extracts exhibited more antioxidant activity than the standard ascorbic acid (50.67%). The results revealed that the leaf extracts of *Chromolaena odorata* contains bioactive compounds having antimicrobial activity and so useful in human medicine.

Keywords: *Chromolaena odorata*, antioxidant, antibacterial activity, phytochemicals, invasive weed.

Introduction

Plants have been an obligatory source of innate products for their relief from illness for many years. The forest is referred to as God's own pharmacy. Many medicinal plants are used in contemporary medicine where they occupy a very significant place, for essential drugs and plants used in traditional system of medicine. Medicinal plants are finding their way into Pharmaceuticals, Nutraceuticals, Cosmetics and Food supplements. The World Health Organization [1] estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs [2]. *Chromolaena odorata* (L.) R. King & H. Robinson formerly called as *Eupatorium odoratum* L. In Nigeria, it is referred to as 'obu inenawa' by the Igbos and 'ewe awolowo' by the Yorubas [3]. *Chromolaena odorata* is a tropical species of flowering shrub in the sunflower family, Asteraceae. It is native to South America and has been introduced to tropical Asia, west Africa, and parts of Australia. It also spreads as a weed in many parts of India. Common names include 'Siam Weed', 'Christmas Bush', 'Devil Weed', 'Camfhur Grass' and 'Common Floss Flower' [4,5]. This plant is also known as wellawel [6]. It is known as 'KAMPU RODDA' in Telugu. It is a rapidly growing perennial with a characteristic aromatic smell and is a multi-stemmed spreading sub-shrub growing 2 to 2.5 m tall in open areas (Plate-1).

Plate:1



It is used as a good wound healer by the native Tribals both for human and cattle in Andhra Pradesh. Medically used in treating

cold, cough, fever, diarrhea, and other skin diseases. Scientific reports had also shown that leaves of plants are major sources of antioxidants, antimicrobials and phyto-chemicals with medicinal values [7,8,9,10]. The most established and discussed aspect of *C. odorata* is its role in wound healing. The wound healing and antibiotic property are recorded by the investigators from the Tribal healers of Addateegala of East Godavari district of Andhra Pradesh where the Tribal Vejjus (Doctors) are treating the injuries of both human and cattle. Extracts from the leaves of *Chromolaena odorata* have been shown to be beneficial for treatment of wounds [10,11]. The phytochemical components viz., Alkaloids, Tannins, Flavonoids and Phenolic compounds found in this plant have medical values. Present study provides the information on preliminary phytochemical screening, antibacterial activity and antioxidant activity of the different solvent extracts of *Chromolaena odorata* leaves.

Materials and methods

Plant extracts preparation

The leaves of *C. odorata* were collected from Forest fringes of Addateegala and authenticated. The leaves were shade dried, packed in paper bags and stored. The dried leaves were crushed and 40g of the pulverized sample was extracted in different solvents like ethyl acetate, acetone, methanol and water (aqueous). The resulting extracts were concentrated in a rotary evaporator, and there after preserved for further use. These extracts were coded as Acetone extract- A, Ethyl acetate Extract-E, Methanol Extract-M, Aqueous Extract Aq.

Phytochemical screening

Reagents used for the different phytochemical tests

The following reagents were prepared and tests were carried out according to standard protocols [12,13].

Mayer's reagent

Mercuric iodide of 1.36 gm was dissolved in 60 ml of water and mixed with a solution containing 5 gm of Potassium iodide in 20 ml of water.

Dragendroff's Reagent

Basic bismuth nitrate (1.7 gm) and tartaric acid (20 gm) were dissolved in 80 ml of water. This solution was mixed with a solution containing 16 gm potassium iodide and 40 ml water.

Fehling's solution- A

Copper sulphate of 34.64 gm was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water and made up to 500 ml.

Fehling's solution- B

Sodium potassium tartarate of 176 gm and sodium hydroxide of 77 gm were dissolved in sufficient water and made up to 500 ml.

Benedicts Reagent

Cupric sulphate (1.73 gm), sodium citrate (1.73 gm) and anhydrous sodium carbonate (10 gm) were dissolved in water and the volume was made up to 100 ml.

Molisch Reagent

Pure α -naphthol of 2.5 gm was dissolved in 25 ml of ethanol.

Liebermann- Burchard Reagent

Acetic acid (5 ml) was carefully mixed under cooling with 5ml concentrated sulfuric acid. This mixture was added cautiously to 50 ml absolute ethanol with cooling.

The following qualitative tests were done to find out the presence or absence of phytochemical constituents like Carbohydrates, Tannins, Steroids, Saponins, Terpenoids, Soluble starch, Flavonoids and Alkaloids.

Test for flavonoids

Ferric chloride test

Two ml of the test solution was boiled with distilled water and filtered. Then, few drops of 10% ferric chloride solution were added to the 2 ml of filtrate. A greenish-blue or violet coloration indicates the presence of a phenolic hydroxyl group.

Shinoda's test

Five grams of each extract was dissolved in ethanol, warmed and then filtered. Small pieces of magnesium chips were then added to the filtrate followed by few drops of conc. HCl. The pink, orange, or red to purple coloration indicates the presence of flavonoids.

Sodium hydroxide test

Extract of 0.2 gm was dissolved in water and filtered. To this, 2 ml of the 10% aqueous sodium hydroxide was added to produce yellow coloration. A change in color from yellow to colorless on addition of dilute hydrochloric acid was the indication for the presence of flavonoids.

Leadacetate test

Extract of 0.5 gm was dissolved in water and filtered. To the 5 ml of each filtrate, 3 ml of lead acetate solution was added. Appearance of a buff-colored precipitate indicates the presence of flavonoids.



Test for alkaloids

Five gms of crude powder was stirred with 1% aqueous HCl on water bath and then filtered. To the 1 ml filtrate, few drops of dragendroff's reagent was added. Orange- Red precipitate was taken as positive. To another 1 ml filtrate, few drops of Mayer's reagent was added and appearance of buff- colored precipitate will be taken as presence of alkaloids.

Test for soluble starch

Crude extract of 0.2 gm was boiled in 1 ml of 5% KOH, cooled and acidified with H₂SO₄. Yellow coloration indicates the presence of soluble starch.

Test for Saponins

Crude powder of 0.5 g was shaken with water in a test tube and it warmed in a water bath. The persistent froth indicates the presence of saponins.

Test for terpenoids

Five gms of crude extract was dissolved in ethanol. To this, 1 ml of acetic acid was added followed by conc. H₂SO₄. A change in color from pink to violet confirms the presence of terpenoids.

Test for steroids

Salkowskii test

In 2 ml of chloroform, 0.2 g of extract was dissolved and added the conc. H₂SO₄. The development of reddish brown color at inter phase indicates the presence of steroids.

Keller-Killiani test

To 0.5 ml of test solution, 2 ml of 3.5% FeCl₃, small amount of glacial acetic acid and 2 ml of conc. H₂SO₄ were added carefully. Appearance of reddish brown ring at inter phase is a positive indication for the presence of steroids.

Liebermann-Burchard test

To 0.2 g of each extract, 2 ml of acetic acid was added and the solution was cooled well in ice followed by the addition of conc. H₂SO₄ carefully. Color development from violet to blue or bluish-green indicates the presence of a steroidal ring (i.e. aglycone portion of cardiac glycoside).

Test for carbohydrates

Molisch's test

Two ml of *Molisch's* reagent was added to the extract dissolved in distilled water and 1 ml of conc. H₂SO₄ was dispensed along the walls of the test tube. The mixture was allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a dull violet color at the inter phase of the two layers indicates the positive test for carbohydrates.

Fehling's test

(for free reducing sugars)

The crude extracts were treated with 5.0 ml of Fehling's solution (A & B) and kept in boiling water bath. The formation of yellow or red color precipitate indicates the presence of free reducing sugars.

Fehling's test

(for Combined Reducing Sugars)

Extract of 0.5 g was hydrolyzed by boiling with 5 ml of dilute hydrochloric acid and the resulting solution neutralized with sodium hydroxide solution. To this, few drops of Fehling's solution were added and then heated on a water bath for 2 minutes. Appearance of a reddish-brown precipitate of cuprous oxide indicates the presence of combined reducing sugars.

Barfoed's test

(for monosaccharide)

In distilled water, 0.5 g of the extract was dissolved and filtered. To 1 ml of the filtrate, 1 ml of Barfoed's reagent was added and then heated on a water bath for 2 minutes. Reddish precipitate of cuprous oxide formation is the positive test for the presence of monosaccharide.

Test organisms used

The crude extracts were tested on four bacterial strains three Gram positive and one Gram negative viz., *Bacillus megaterium* MTCC-428, *Bacillus subtilis* MTCC-441, *Staphylococcus aureus* MTCC-737 and *Proteus mirabilis* MTCC-425.

Antibacterial screening

The crude extracts were screened for antibacterial activity by using agar well diffusion method[14]. Bacterial suspensions were prepared by using 24 hours old bacterial cultures. Into the sterile Petri plates, 1 ml of the bacterial suspension was added followed by molten state nutrient agar medium and mixed well. After complete solidification, wells were bored with sterile cork borer of 6 mm diameter. Then, the wells were filled with 100 µl of the extract sample prepared by dissolving 100 mg of extract in 1 ml of DMSO. The plates were incubated at 37°C for 24 hrs. After incubation, the diameter of the zone of inhibition was measured. For each sample and bacterial species, triplicates were maintained. Streptomycin standard

antibiotic was used as positive control in the concentration of 10µg/ml DMSO.

In vitro antioxidant assay

DPPH (2, 2-diphenyl-1-picryl hydrazyl) Free radical scavenging activity

The antioxidant activity of the extracts was determined according to the method of [15]. Different solvent extracts were prepared in the concentration of 100 µg/ml in DMSO. To the plant extract, 4 ml of the 0.004% (w/v) DPPH solution prepared in methanol was added and the reaction mixture was kept for incubation in dark for 30 minutes. Ascorbic acid was used as standard. The absorbance was measured at 517 nm. The DPPH scavenging activity (%) was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = [(A_0 - A_s) / A_0] \times 100,$$

Where, A_0 is the absorbance of the control

A_s is the absorbance of the plant sample

Results and discussion

In the present study, different solvent extracts of *chromolaena odorata* leaves were tested for the screening of phytochemical constituents, antibacterial activity and antioxidant activity. Table-1 displayed the phytochemicals present in different solvent extracts.

Table -1. Phytochemical analysis of *Chromolaena odorata* leaf extracts in different solvents

S.No.	Phytochemical	A	E	M	Aq
1.	Carbohydrates	--	--	--	+
2.	Tannins	--	+	--	+
3.	Steroids	+	+	--	--
4.	Terpenoids	--	--	--	--
5.	Saponins	--	+	--	+
6.	Flavonoids	+	+	+	+
7.	Alkaloids	+	+	+	+
8.	Soluble starch	--	--	--	--

A - Acetone; E - Ethyl acetate; M - Methanol; Aq -Aqueous

From a single test we cannot conclude the nature of secondary metabolites. So, for more experimental evidence more than one color test was conducted in most of the cases. Ethyl acetate and aqueous extracts were positive for most of the phyto-constituents viz., tannins, steroids and saponins. Flavonoids and alkaloids were present in all the extracts. All the extracts tested for the terpenoids and soluble starch were negative. Figure-1 shows the results regarding antibacterial activity of different extracts. The pictures of inhibition zones of some solvent extracts against different bacteria are given in plate-2.

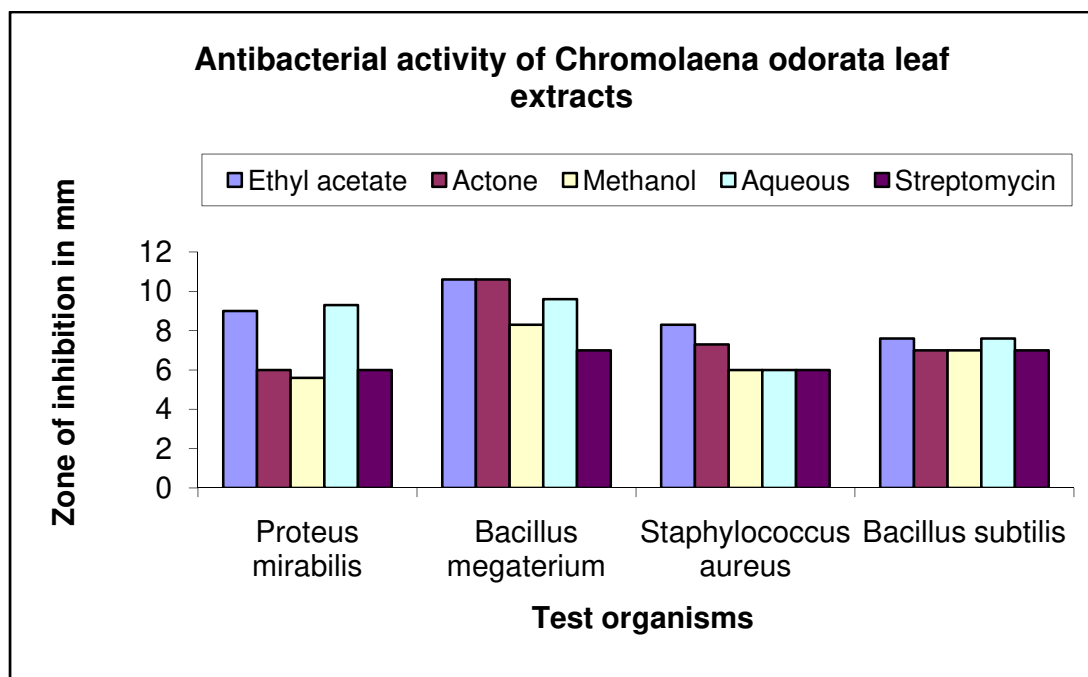


Figure-1. Antibacterial activity of *Chromolaena odorata* leaf extracts in different solvents

Plate-2



A) Acetone extract against *B. megaterium*
 C) Ethylacetate extract against *S. aureus*

B) Aqueous extract against *B. megaterium*
 D) Aqueous extract against *P. mirabilis*

Ethyl acetate extract showed relatively larger zone of diameter than the streptomycin (standard antibiotic) against all tested organisms. Acetone extract exhibited larger zones against *Bacillus megaterium* and *Staphylococcus aureus* and equal zone of diameter against *Proteus mirabilis* and *Bacillus subtilis* than the positive control. Methanol extract of *Chromolaena odorata* leaf showed good antibacterial activity against *Bacillus megaterium*, *Staphylococcus aureus* and *Bacillus subtilis*. Against *Proteus mirabilis*, *Staphylococcus aureus* and *Bacillus megaterium*, the aqueous extract showed the best antibacterial activity than the positive control.

Figure-2 shows the results of antioxidant activities of different solvent extracts. The ethyl acetate and aqueous extracts showed best results than the standard antioxidant, ascorbic acid.

Most of the phytochemical surveys carried out on this *Chromolaena odorata* plant were concentrated on testing the flavonoids, since flavonoids comprise a vast array of biologically active compounds that are ubiquitous in plants, many of which

have been used in traditional eastern medicine for thousands of years [16]. Free radical mechanism is one of the most important mechanisms of liver damage. Metabolism of certain drugs like paracetamol produce free radicals, which cause liver damage [17]. Medicinal plants are important source of antioxidants [18,19]. The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits seeds, roots and bark [19,20]. Plant based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials needs to occur. Antimicrobials of plant origin have enormous therapeutic potential [21,22]. Recently, much attention has been directed toward plant extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms [22,23,24].

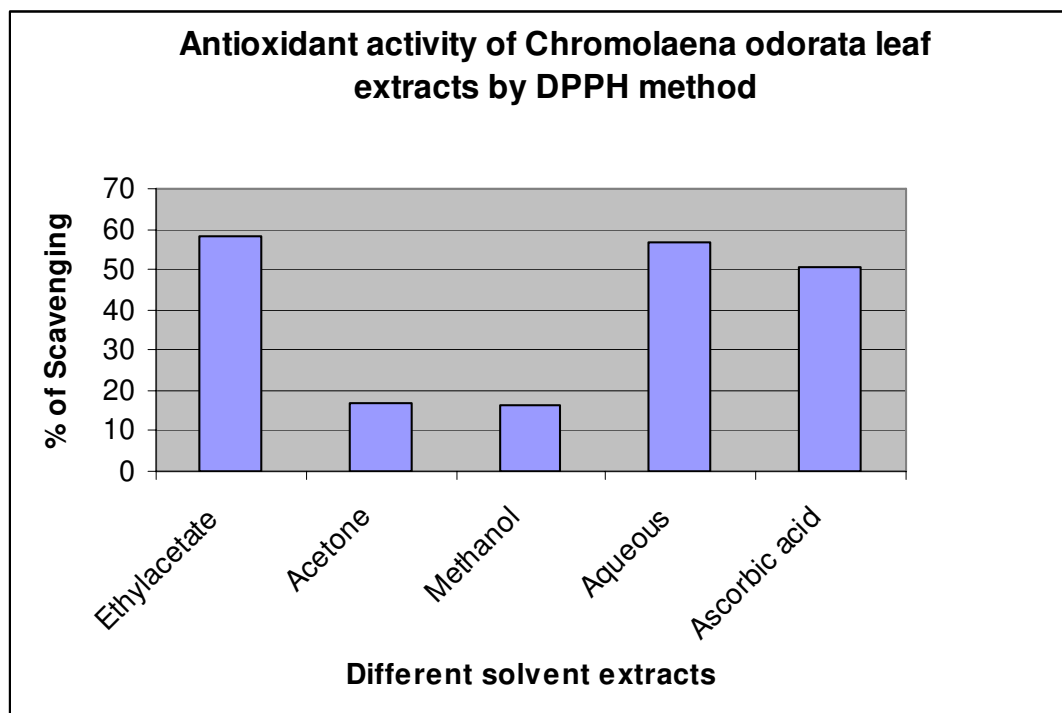


Figure-2. Antioxidant activity of *Chromolaena odorata* leaf extracts by DPPH Scavenging method

Conclusion

This study indicates that leaf extracts of *chromolaena odorata* possesses bioactive metabolites, free radical scavenging activity and antibacterial activity which can be explored further in the field of human medicine.

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Acknowledgements

The authors are grateful to the Head, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar-522 510, Guntur District, & Dr. G. Gangaraju, Chairman Laila Nutra R & D Center, Vijayawada, Andhra Pradesh, India for support.

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