Antioxidant And Antimicrobial Activity Study of Chromolaena Odorata Root Extract

Kandakatla Tejasri[1,] M. Rakesh[2]
[1,2] *Department of Pharmacology,*
Jayamukhi College of Pharmacy, Narsampet, Warangal-506332, Telangana, India

***Abstract*—Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and endogenous antioxidant defenses, plays a pivotal role in the pathogenesis of numerous chronic diseases. This condition is significantly exacerbated during microbial infections and can drive the initiation and progression of cancer by damaging essential macromolecules, including DNA, proteins, and lipids. Antioxidants Act as the brakes, stopping the stress from hurting you. The current study has developed to assess the antioxidant and antimicrobial effect of ethanolic and aqueous extract of *chromolaena odorata* roots. Aqueous, ethanolic, and chloroform extracts were assessed using DPPH radical scavenging, nitric oxide scavenging, Hydroxyl radical scavenging activity, and aqueous and ethanolic extracts are assayed using Disc diffusion and Cup Plate Method against standard antibiotic control sample. All the extracts showed a positive correlation with antioxidant activity, antibacterial sensitivity test revealed that ethanolic extracts of *Chromolaena odorata roots* at 50%, 20%, 10% and 5% concentrations showed antibacterial activities against Staphylococcus aureus and Bacillus cereus in both Disc diffusion assay and Cup Plate Method. These results demonstrate that the roots of *Chromolaena odorata* exhibit potent antioxidant and antimicrobial activities, underscoring their potential as a viable natural reservoir for the development of novel antimicrobial therapeutics.**

***Index Terms*—*Chromolaena odorata*, Antioxidant activity, Antimicrobial, DPPH assay, nitric oxide scavenging, Hydroxyl radical scavenging activity, Disc diffusion assay**

I. INTRODUCTION

The majority of present diseases are reported to be due to the imbalance of pro-oxidant and the antioxidant homeostatic phenomenon in the body. Pro-oxidant conditions prevail only when there is excessive production of free radicals due to oxidative stress or when the defense mechanism of the body weakens; result in reducing scavenging/quenching ability because of the deficiency of antioxidant. Oxidative stress and microbial infection share a reciprocal, double-edged relationship where the host immune system generates reactive oxygen species as a toxic weapon to kill invading pathogens, while the resulting oxidative imbalance can simultaneously cause collateral damage to host tissues and trigger microbial counter-defences

Free radicals can irreversibly damage the cell and vital molecules such as nucleic acids, lipids and proteins. This is believed to play a central role in the aging process and in disease progression (1). Traditional and indigenous medical system of treatment, in India. (Ayurveda), China and other countries are based on dietary and medicinal plants for the maintenance and promotion of health, and in treating ailments (2). They have important roles in bio-prospecting of new medicines from medicinal plants, which are also rich sources of antioxidants. Though, modern medicine system is at the forefront yet, current estimate indicates that about 80% of people in developing countries still rely on traditional medicine-based largely on various species of plants and animals for their primary healthcare (3).

This study evaluates the in-vitro antioxidant and antimicrobial potential of *chromolaena odorata* root extracts specifically aqueous, ethanolic preparations to validate its ethnomedical use and identify its efficacy as a natural source for combating oxidative stress and microbial infection.

II. MATERIAL AND METHODS

CHEMICALS AND REAGENTS:

1,1-diphenyl-2-picrylhydrazyl (DPPH), Ascorbic acid, Methanol, Sodium nitroprusside, Phosphate buffer (pH 7.4), Streptococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa bacterial strains,Streptomycin, Mueller Hinton Agar.

COLLECTION OF PLANT MATERIAL:

The roots of plant were collected from near places of Narsampet, Warangal district. The preliminary evaluation was done. Then the roots are washed properly under the tap water to remove mud and dust particles. Shade drying is the most acceptable form of drying which involves less exposure to heat, and there are less chances of chemical alteration. After the complete drying of the roots smash and grind them with the help of grinder. Now the powder is used for the extraction process.

PREPARATION OF EXTRACTS:

The powdered plant material underwent successive solvent extraction using a Soxhlet apparatus. The process began with petroleum ether (60–80°C), followed sequentially by chloroform, ethanol, and distilled water, with each cycle lasting 72 hours at temperatures maintained below the respective boiling points. Each resulting extract was filtered through Whatman No. 1 paper, concentrated under vacuum to ensure complete solvent removal, and stored in sterile containers at 4°C for subsequent analysis (4).

IN-VITRO ANTIOXIDANT ACTIVITY

DPPH RADICAL SCAVENGING ASSAY

The antioxidant capacity of Paspalum paniculatum root extracts (aqueous, ethanolic, and chloroform) was determined via the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. A 0.0033% (w/v) DPPH solution was prepared in methanol and protected from light. Stock solutions (1000 µg/mL) of each extract were diluted to a working concentration range of 25–200 µg/mL, with ascorbic acid serving as the reference standard. For the reaction, 1 mL of each sample or standard was combined with 5 mL of the DPPH reagent and incubated at 37°C for 20 minutes in the dark. Absorbance was subsequently measured at 516 nm using a UV–Visible spectrophotometer against a methanol blank, with the DPPH-solvent mixture serving as the negative control [5].

The percentage of radical scavenging activity was calculated using the following equation:

$$\%\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of } Control} \times 100$$

The IC_{50} value was determined from the plot of percentage inhibition versus concentration.

IN VITRO ANTIMICROBIAL ASSAY

DISC DIFFUSION ASSAY

The antibacterial activity of *Chromolaena odorata* root extracts (ethanolic and aqueous) was evaluated using the disc diffusion method against four bacterial strains. Inocula were prepared from 18-hour cultures and standardized to a 0.5 McFarland turbidity (1.5 \times 10^8 CFU/mL) using sterile saline. A 500 µL aliquot of the bacterial suspension was uniformly spread onto Mueller-Hinton Agar (MHA) plates. To enhance extract solubility and diffusion, a diluent of 10% dimethyl sulfoxide (DMSO) and 0.5% Tween 80 was utilized. Extracts were prepared at concentrations of 5%, 10%, 20%, and 50%, then sterilized via a 0.22 µm membrane filter. Under aseptic conditions, sterile Whatman No. 5 filter paper discs (6 mm diameter) were impregnated with 50 µL of each extract concentration and placed on the inoculated agar. Discs moistened with the diluent served as vehicle controls, while standard antibiotic discs were used as positive references. Plates were sealed with parafilm, allowed to pre-diffuse for 30 minutes at room temperature, and incubated at 37°C for 18 hours. Antibacterial efficacy was determined by measuring the zones of inhibition (ZOI) using a vernier caliper, with all tests performed in triplicate (6).

STATISTICAL ANALYSIS

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). IC_{50} and LD_{50} values were calculated using regression analysis from dose–response curves. (7)

III. RESULTS

IN-VITRO ANTIOXIDANT ACTIVITY- DPPH RADICAL SCAVENGING ASSAY

The DPPH free radical scavenging assay revealed that the chloroform, ethanol, and aqueous root extracts of *Chromolaena odorata* possess significant antioxidant potential. As shown in Table 1, all extracts demonstrated concentration-dependent inhibitory activity, with radical scavenging efficacy increasing proportionally with extract concentration.

Among the tested extracts, the ethanolic extract demonstrated the highest antioxidant activity, followed by the aqueous and chloroform extracts. The IC_{50} values of chloroform, ethanolic, and aqueous extracts were found to be 128.76, 56.75, and 103.25 µg/mL, respectively, whereas the standard ascorbic acid exhibited an IC_{50} value of 38.17µg/mL. These results suggest that the ethanolic extract possesses comparatively stronger antioxidant potential.

Conc. (µg/ml)	% Inhibition			
	ASA	Chloroform extract	Ethanol extract	Aqueous extract
25	19.54	7.04	13.82	10.15
50	37.75	15.32	28.56	13.52
75	44.34	22.32	32.13	18.64
100	57.56	29.22	45.45	25.43
125	65.53	37.27	53.64	33.74
150	74.78	41.14	61.35	45.16
175	81.92	48.17	69.89	51.54
200	89.79	51.21	80.18	58.85
IC_{50}(µg/ml)	38.17	128.76	56.75	103.25

DISC DIFFUSION ASSAY

According to the findings of the present study, *Chromolaena odorata roots* ethanolic extracts have varied degrees of antibacterial activity in terms of zone of inhibition against tested gram positive and gram-negative bacteria except Pseudomonas aeruginosa. (Table 2).

Zone of inhibition (mm)				
Ethanolic Extract				
Group	Staphylococcus aureus	Bacillus cereus	Escherichia coli	Pseudomonas aeruginosa
CO A (50%)	11.33±0.33	10.67±0.33	0.00	0.00
CO B (20%)	10.67±0.33	8.67±0.33	0.00	0.00
CO C (10%)	9.67±0.33	8.33±0.33	0.00	0.00
CO D (5%)	9.33±0.67	8.00±0.00	0.00	0.00
Aqueous extract				
CO A (50%)	9.00±1.00	8.67±0.33	10.67±0.33	0.00
CO B (20%)	0.00	0.00	0.00	0.00
CO C (10%)	0.00	0.00	0.00	0.00
CO D (5%)	0.00	0.00	0.00	0.00
streptomycin	17.33±0.88	15.13±0.33	11.76±0.33	19.00±0.58

IV. DISCUSSION

Oxidative stress arises from a critical disparity between the generation of reactive oxygen species (ROS) and the endogenous antioxidant systems designed to neutralize them. In light of this, there is an urgent need to identify and characterize natural products that possess dual antioxidant and antimicrobial potential, offering a sustainable and effective strategy to mitigate cellular damage while simultaneously combating resistant pathogens.

From this perspective, the current study has developed to assess the antioxidant and antimicrobial effect of ethanolic and aqueous extract of *chromolaena odorata* roots. The study utilized multiple invitro assay methods for antioxidant and antimicrobial activity. Initially the phytochemical analysis has done for four root extracts i.e., pet ether, chloroform, ethanol and aqueous solvents. As the observations from the results the ethanol extract has shown more percentage of phytochemicals compared to the remaining that is followed by aqueous extract, chloroform and pet ether. Consistent with the results of this study, ethanol proved to be the most efficient solvent for extraction, yielding a superior concentration of flavonoids and alkaloids. This confirms that its intermediate polarity allows for a more comprehensive extraction of the plant's chemical profile than highly polar or non-polar alternatives. The antioxidant efficacy of alkaloids is largely due to the presence of nitrogen atoms in their heterocyclic rings, which can donate electrons to neutralize Reactive Oxygen Species (ROS) without becoming reactive themselves. Flavonoids exert antioxidant activity by donating a hydrogen atom from their hydroxyl groups to unstable free radicals, effectively neutralizing them and forming a more stable phenoxyl radical. Tannins contribute to the stability of the extract by forming complexes with proteins and metal ions, thereby preventing oxidative degradation and inhibiting pro-oxidant metal-catalyzed reactions. Saponins identified in the extract contribute to antioxidant activity by reacting directly with free radicals and preventing the chain reaction of lipid peroxidation within cell membranes. With this mechanism all these compounds possess antioxidant property.

Phytochemical screening serves as a critical first step in unlocking the therapeutic secrets of the plant, providing a qualitative map of the bioactive molecules present. In the present study, *chromolaena odorata* root extracts exhibited a broad spectrum of phytochemicals: alkaloids, carbohydrates, flavonoids, steroids and triterpenoids, amino acids, tannins, saponins, fixed oils and fats, and glycosides were present, particularly in ethanolic extracts

Based on the rich diversity of secondary metabolites identified during the preliminary screening specifically the presence of phenolic compounds and flavonoids the extract was subjected to further quantitative analysis to evaluate its in vitro antioxidant potential

The two extracts (ethanol and aqueous) were selected for the invitro antioxidant (DPPH free radical scavenging activity, Hydroxyl radical scavenging activity, Nitric oxide radical scavenging assay,) and antimicrobial (Disc diffusion assay and Cup Plate Method). From the antioxidant activity result, it is found that two extracts *chromolaena odorata* roots showed potent antioxidant activity. But ethanolic extract showed more antioxidant activity as compared to and water extract. The antioxidant activity of extracts might be due to the presence of chemical constituent/s like saponins, phenolic compounds and alkaloids. Result of the present study on antibacterial sensitivity test revealed that ethanolic extracts of *Chromolaena odorata roots* at 50%, 20%, 10% and 5% concentrations showed antibacterial activities against Staphylococcus aureus and Bacillus cereus in both Disc diffusion assay and Cup Plate Method.

V. CONCLUSION

Chromolaena odorata roots possesses significant in-vitro antioxidant activity and antimicrobial activity specifically in the ethanolic extract through multitargeted approach, suggesting that these extracts may serve as effective natural alternatives for antioxidant and antimicrobial therapy.

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