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Antimicrobial Investigation of *Parthenium Hysterophorus linn* Leaf Extract against Plant Pathogenic and Beneficial Microorganisms

Phatangare A. T, Shinde. H. S and V. A. Mane
Department of Post-Harvest and Food Biotechnology,
Department of Plant Biotechnology,
K. K. Wagh College of Agricultural Biotechnology, Nashik. 422003, India

***Abstract* – An extract of *Parthenium Hysterophorus Linn* were screened for phytochemical analysis and antimicrobial assay against beneficial bacteria like *Azotobacter spp., Rhizobium spp., Pseudomonas spp., Azospirillum spp* and plant pathogenic bacteria like *Xanthomonas oryzae, Agrobacterium tumefaciens, Erwinia amylovora* and *Xylella fastidiosa.* The solvent used for extract preparation was methanol. Phytochemical analysis of leaf extract shows presence of Alkaloids, phenols, carbohydrates, glycosides, Terpenoids, steroids and proteins. Agar well diffusion method was used for study of antimicrobial activity of leaf extract. Standard antibiotic (Erythromycin) and 5% DMSO were used as a positive and negative respectively. Parthenium leaf extract shows strong antimicrobial activity against plant pathogenic microorganism whereas beneficial bacteria are resistant to parthenium leaf extract.**

***Keywords* – *Parthenium Hysterophorus Linn*, Azotobactor Spp, Antimicrobial Activity, Phytochemical Analysis, Agar Well Diffusion Method.**

I. INTRODUCTION

Parthenium Hysterophorus Linn (Family-Asteraceae)*Parthenium Hysterophorus Linn* is king of Weed and in India is commonly known as *Congress grass, Congress weed, Carrot Weed, Bitter Weed, Star Weed, White Top, Chatak Chandni, Ramphool* and *gajar Ghas.*[1] It is an exotic weed that was accidentally introduced in India in 1955 through imported food grains.[2] Now it was considered as one of the most toxic weed. *Parthenium Hysterophorus Linn* is used in the treatment of fever, anaemia and heart troubles.[3] In this study, we had evaluated the phytochemical analysis and antimicrobial activity of methanol extract of *Parthenium Hysterophorus Linn* Leaves against plant pathogenic bacteria and beneficial bacteria.

II. MATERIALS AND METHOD

Plant material-

Fresh leaves of *Parthenium Hysterophorus Linn* were collected from Puria Park *i.e* Agricultural land of K. K. Wagh College of Agricultural Biotechnology, Nashik, India. These fresh leaves were washed thoroughly with tap water and then by distilled water and then shade dried for 2-3 days. The dried leaves were powdered with the help of mixer and grinder, and 10 gm of powder used for the extract preparation.

Preparation of Extract-

For the preparation of plant extract 20 gm of leaf powder was extracted in a Soxhlet Apparatus using 200 ml of methanol solvent. The extract were concentrated using rotary evaporator. The extract obtained were weighed and kept at 4^0C. 15 mg of solvent residue was dissolved in 1 ml of DMSO (5%) as a solvent and were used as the test extracts for antimicrobial activity.[4]

Phytochemical analysis-

The phytochemical analysis was carried out on the methanol extract using standard procedures to identify the phytochemical constituents.[5]

a) *Alkaloids-* 0.5 g of each sample were dissolved with 5 ml of 2 N HCl and filtered. Filtrate was treated with Dragendroff's reagent. Formation of red precipitate indicates the presence of alkaloid.

b) *Phenols-* Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

c) *Carbohydrates-* To 1 ml of the filtrate, 5 ml of Benedict's reagent were added. The mixture was heated. Appearance of red precipitate indicated the presence of reducing sugar.[6]

d) *Glycosides-* 0.5 g of each extract was stirred with 10 ml of boiling distilled water. This was filtered and 2 ml of the filtrate hydrolyzed with a few drops of concentrated HCL and the solution rendered alkaline with a 5 drops of ammonia solution. 5 drops of this solution was added to 2 ml of Benedict's qualitative reagent and boiled. Appearance of reddish brown precipitate showed the presence of glycosides.

e) *Terpenoids-* 5 ml of extract was mixed in 2 ml of chloroform and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown colour formation shows positive results for the presence of terpenoids.[7]

f) *Steroids-* The extract was mixed with 2 ml of chloroform and concentrated sulphuric acid was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

g) *Proteins (Millon's test)-* Small portion of the extract when mixed with 2 ml of millon's reagent, white precipitation appeared which turned red upon gentle heating that confirmed the presence of protein.[8]

Isolation and maintenance of pathogen-

Isolation of beneficial microorganisms was carried out using rhizospheric soil. For the isolation beneficial like *Azotobacter spp., Rhizobium spp., Pseudomonas spp.,*

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*Azospirillum spp*we used selective mediums as Jensen's medium, Rhizobium medium, King's B Agar medium and Azospirillum medium w/0.17% Agar respectively.[9]For isolation of pathogenic microorganisms from different sources we used different media as for isolation of *Xanthomonas oryzae, Agrobacterium tumefaciens, Erwinia amylovora* and X*ylella fastidiosa* we used yeast extract caleium carbonate (YDC) Agar medium, MGY medium, Erwinia amylovora selective medium and PD3 medium respectively.[10]

Antimicrobial Assay-

Antimicrobial activity of solvent extract methanol was determined by Well Diffusion Method on Nutrient Agar medium.[11]A well with diameter 5mm was made using sterile cork borer and inoculums of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50□g each of solvent extracts were poured in the wells of the inoculated plates. The plates were incubated for 24 hrs at 37^0C and zone of inhibition if any around the wells were measured in mm.

III. Result

After extraction of *parthenium* leaf extract in Soxhlet using methanol as a solvent, extract was subjected to various quantitative phytochemical analysis result of phytochemical analysis is summarized in Table 1. The zone of inhibition produced by parthenium leaf extract against beneficial microorganisms and plant pathogenic microorganisms is observed and noted in Table 2 and Table 3 respectively. Standard antibiotic (Erythromycin) was used as a positive control and solvent preservative DMSO (5%) was used as a negative control.

Table 1: Phytochemical analysis of methanol extract of *Parthenium hysterophorus linn*

S.No.	Phytochemicals	*Parthenium Hysterophorus linn*
1	Alkaloids	+
2	Phenols	+
3	Carbohydrates	+
4	Glycosides	+
5	Terpenoids	+
6	Steroids	+
7	Proteins	+

Table 2: Antimicrobial activity of methanol extract of *Parthenium hysterophorus linn* against different beneficial bacteria

S. No.	Bacterial cultures	*Parthenium hysterophorus linn* (in mm)	Erythro-mycin (in mm)
1	*Azotobacter spp.*	9	16
2	*Rhizobium spp.*	7	17
3	*Azospirillum spp.*	8	13
4	*Pseudomonas spp.*	11	12

Table 3: Antimicrobial activity of methanol extract of *Parthenium hysterophorus linn* against different pathogenic bacteria.

S. No.	Bacterial culture	*Parthenium hysterophorus linn* (in mm)	Erythromycin (in mm)
1	*Xanthomonas oryzae*	14	15
2	*Agrobacterium tumefaciens*	12	13
3	*Erwinia amylovora*	13	12
4	*Xylella fastidiosa*	17	18

IV. Discussion

In the present study, we have carried out the phytochemical analysis of methanol extract of *Parthenium hysterophorus* linn leaves.[12] Alkaloids, Phenols, Carbohydrates, glycosides, Terpenoids, Steroids and proteins are present in extracts all these biochemical compounds are biologically active compunds.

In antimicrobial study we have investigated that *parthenium* leaf extract shows antimicrobial activity against plant pathogenic microorganisms like *Xanthomonas oryzae, Agrobacterium tumefaciens, Erwinia amylovora* and X*ylella fastidiosa* by forming zone of inhibition of 14mm, 12mm, 13mm and 17mm respectively whereas beneficial bacteria like *Azotobacter spp, Rhizobium spp, Pseudomonas spp* and *Azospirillum spp* shows resistant to *parthenium* leaf extract by forming zone of inhibition of 9mm, 7mm, 8mm and 11mm respectively. From all these study we can conclude beneficial microorganism has acquired resistance to *parthenium* so we can use the *Parthenium* extract to control various diseases of plant without giving harm to beneficial microorganisms.

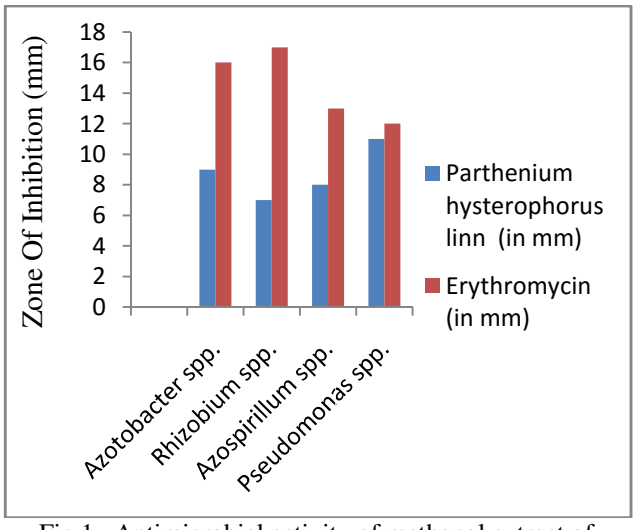

Fig.1. Antimicrobial activity of methanol extract of *Parthenium hysterophorus linn* against different beneficial bacteria

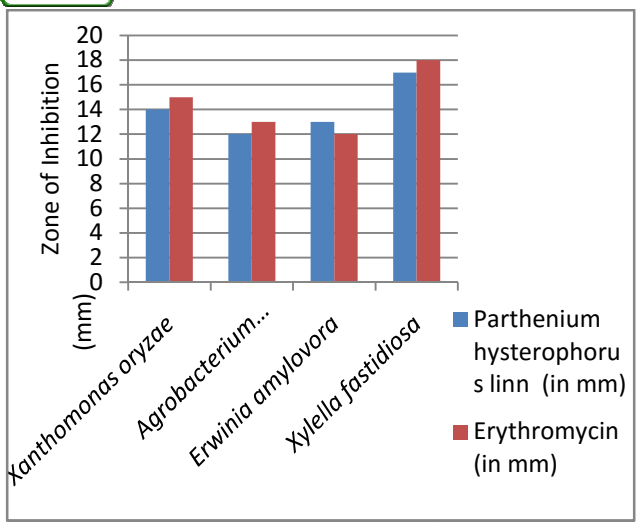

Fig.2. Antimicrobial activity of methanol extract of *Parthenium hysterophorus linn* against different pathogenic bacteria

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AUTHOR'S PROFILE

Ashutosh Trimbak Phatangare
is undergraduate student in K. K. Wagh CABT, Nashik College during his hands on training he worked on antimicrobial properties of *Parthenium.* He was born in Ahmednagar, Maharashtra on 18/02/1993.

Harshraj Subhash Shinde
is working as a Assistant professor in Department of post harvest, K. K. Wagh CABT, Nashik from past on and half year He has qualified ARS- NET in agricultural Biotechnology in 2014. He was working as a researcher in Seoul national University, South Korea. He was Born in Pandharpur (Maharashtra) on 19/06/1988.

Vidya Anirudha Mane
is working as a Assistant professor in Department of Plant Biotechnology K. K. Wagh CABT, Nashik from past three and half years. She has published seven no of research articles in peer reviewed journals and two review articles. She was Born in Solapur (Maharashtra) on 26/07/1987.