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Mass Multiplication, Phytochemical, Antibacterial and Molecular Analysis of an Important Medicinal Plant *Achyranthes Aspera* Linn

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Abstract: *Achyranthes aspera Linn., (Amaranthaceae) is an important medicinal herb found as a weed throughout India. This plant has been used by Ayurvedic and Yunani practitioners to treat leprosy, asthma, piles, wounds, insects and snake bite, kidney stone, diabetes, dermatological disorders, gynecological disorders, fever, malaria, pneumonia, tooth ache, gonorrhea, rabies etc. The plant is reported to be used as antimicrobial, anti inflammatory, antipyretic and hepatoprotective. The present investigation proved that the bulging of explants and induction of callus was observed on 10th day of culture. Callus formation was obtained within a period of 2 weeks in media supplemented with IAA and BAP. The aqueous, ethanol, methanol and chloroform leaf extracts of Achyranthes aspera Linn. Were evaluated for antibacterial activity against Escherichia coli, Staphylococcus aureus, Pseudomonas species and Bacillus subtilis. All the extracts of this plant apparently showed [1] considerable inhibitory activity against all tested microorganisms by agar well diffusion method. All the extracts of Achyranthes aspera Linn. Showed maximum zone formation against Escherchia coli [2], and Staphylococcus aureus than the chloroform and aqueous extracts [3]. Achyranthes aspera can be effectively used in treatment of diseases caused by Escherchia coli and [4] Staphylococcus aureus. The antibacterial properties of Achyranthes aspera is due the presence of phytochemical constituents like carbohydrates, proteins and amino acids, alkaloids, steroids and phenolic compounds. DNA isolation is a procedure to collect DNA for subsequent molecular analysis. The total genomic DNA was extracted by using cetyltrimethyylammonium bromide (CTAB) method (Doyle. 1987) from young leaf tissue, Achyranthes aspera revealed a significant scope to [5] develop a novel broad spectrum of antimicrobial drug formulation.*

Keywords: *phytochemical, in vitro analysis, medicinal plant, antibacterial activity*

1. INTRODUCTION

Knowledge of herbs has been handed down from generation to generation for thousands of years [1].Herbal drugs constitute a major part in all traditional systems of medicines. Herbal medicine is a triumph of popular therapeutic diversity. Plants above all other agents have been used for medicine from time immemorial because they have fitted the immediate personal need, are easily accessible and inexpensive [2].In the recent past there has been a tremendous increase in the use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. An upward trend has been observed in the research on herbals. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species [3]. According to the WHO more than 80 % of the world's population relies on traditional herbal medicine for their primary health care [4]. Plants continue to serve as possible sources for new drugs and chemicals derived from various parts of plants [5]. In recent time there has been a marked shift towards herbal cures because of the pronounced cumulative and irreversible reactions of modern drugs. However, due to over population, urbanization and continuous exploitation of these herbal reserves, the natural resources along with their related traditional knowledge are depleting day by day [6]. In the present era of drug development and discovery of newer drug molecules many plant [1] products are evaluated on the basis of their traditional uses. One of the many plants which are being

evaluated for their therapeutic efficacies is *Achyranthes aspera* which is commonly known as Latjeera [2] (Hindi) and Rough Chaff tree (English). It is an erect or procumbent, annual or perennial herb, 1-2m [3] in height, often with a woody base, commonly found as a weed of waysides, on roadsides [7, 8, 9]. Although it has many medicinal properties, it is particularly used spermicidal [10], antipyretic [11] [4] and as a cardiovascular agent [12][5].

2. MATERIALS AND METHODS

2.1. In Vitro Studies

The present investigation on *in vitro* propagation of *Achyranthes aspera* was attempted in the plant [1] tissue laboratory of the PG and Research Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore. *Achyranthes aspera* leaves were collected from Erode district and used [2] for the entire course of study.

The explants were washed thoroughly in running tap water for 30 minutes and placed in detergent solution (Teepol-5% v/v) for 10 minutes. Then explants were washed in running tap water until the removal of last traces of detergent solution. Then the explants were transferred in front of laminar air flow and disinfected with 0.1% aqueous mercuric chloride for a period of 10 minutes. Finally, they were washed several times with distilled water. The disinfected plantlets were taken for inoculation.

Nutrient support must be essential for optimal growth of a plant *in vitro.* The nutritional supplement in the medium may vary with species. Selection and preparation of particular media is one of the important steps in the *in vitro* studies. The nutrient media consists of inorganic nutrient, carbon sources and organic supplements. In addition, vitamins and growth regulators were also added to this media. In the present study, MS [13] basal medium was used. The autoclaved medium in the culture bottles were cooled and allowed to solidify and it was stored in dark for future use. The inoculations were done after four days to ensure that the bottles were free from contamination.

2.1.1. Culture Condition

All the cultures were maintained in the culture room at a temperature of 25±2 º C and relative humidity of 60-70 %. The cultures were kept under white light at intensity of 2000 lux provided from fluorescent lamps, with 14 hours photoperiodic duration.

2.2. Antibacterial Activity

2.2.1. Culture Media

Nutrient agar media was used to test the antibacterial activity of the leaf extracts of *Achyranthes aspera.* Bacterial strains - *Escherichia coli, Staphylococcus aureus, Pseudomonas* species and *Bacillus subtilis* were inoculated on nutrient broth, left in shaking conditions at 37ºC for [3] 24 hours. This served as overnight culture. The antibacterial activity was tested against various leaf extracts of *Achyranthes aspera* - aqueous, ethanol, methanol and choloroform. About 15-20 ml of nutrient agar medium was poured in the sterilized petri dish and allowed to solidify. One drop of bacterial strains was spread over the medium by a sterile cotton swab. Wells of 6mm in diameter and about 2 cm was punctured in the culture medium using sterile cork borer. About 25µl, 50 µl, 75 µl, 100 µl of plant extracts was added to the wells. Plates were incubated at 37ºC for 24 hours. Antibacterial activities were evaluated by measuring the diameter of zone of inhibition.

2.3. Phytochemical Analysis

2.3.1. Qualitative Screening

Different qualitative chemical tests can be performed for establishing profile of [10] aqueous extract for its chemical compositions.

2.4. Extraction of Total DNA from Plant Tissue

Total DNA extraction of the leaf sample was carried out by CTAB Method (Doyle, 1987) [1]

2.5. Restriction Fragment Length Polymorphism (RFLP)

RFLP is a technique that exploits homologous DNA sequence. In RELP analysis, DNA sample is broken in to pieces by restriction enzymes and the resulting fragments are separated to their

length by gel electrophoresis. It is a variation in the DNA sequence of a genome. Analysis of RFLP variation was an important tool in genome mapping, localization of genetic disease genes, determination of risk for a disease, genetic fingerprint and paternity testing. Once a diseased gene was localized, RELP analysis of other families could reveal who were at risk for the disease or who were likely to be carriers of the mutant gene. Restriction enzymes are used to cleave identical DNA molecules as a consequence of allelic difference arises during evolution since mutation can produce DNA fragments of different sizes.

2.6. Fourier Transform Infrared Spectroscopy (FTIR)

It is a technique which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman Scattering of a solid, liquid or gas. An FTIR spectrometer simultaneously collects spectral data in a wide range. It is most useful for identifying chemicals that are either organic or inorganic. FTIR can be used to identify chemicals from spills, paints, polymers, coatings, drugs and contaminants. It is also used to determine the different components in the sample.

FT-IR is perhaps the most powerful tool for identifying types of chemicals bonds (functional groups). The wave length of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infra red absorption spectrum, the chemical bonds in a molecule can be determined.

Dried powder of ethanolic extract of *Achyranthes aspera* plant was considered for instrumental analysis. For the FT-IR study dried powder of ethanolic extract was used. 10mg of the plant was [2] encapsulated in 100mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of *Achyranthes aspera* was treated for FT-IR spectroscopy (Shimadzu, IR Affinity 1, Japan). Scan range: from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

3. RESULTS

3.1. In Vitro Regeneration

Bulging of explants and induction of callus was observed on 10^{th} day of culture. Callus formation was obtained within a period of 2 weeks in media supplemented with IAA and BAP.

The tap root retains cells in which it possess momentum due to the presence of maximum number of physiologically active meristematic cells. As the explants absorbs nutrients it make continuous nutrient gradient among different cells of explant on basis of observation. As a result the cell differentiates and dives asynchronously to give callus tissues. Callus obtained after incubation can be further proliferated and maintained by sub culturing in auxin rich medium. Callus cultured are needed to be sub cultured in every 3-5 weeks in view of cell growth, nutrient depletion and media drying. Regeneration of shoots and roots from callus requires subculture into root induction and shoot induction media.

Proliferation of multiple shoots was observed with high frequency from nodal segments and shoot tips with different combination of cytokinin. The high rate of shoot root multiplication and post hardening survival indicates that this protocol could be easily adopted for commercial large scale protection.

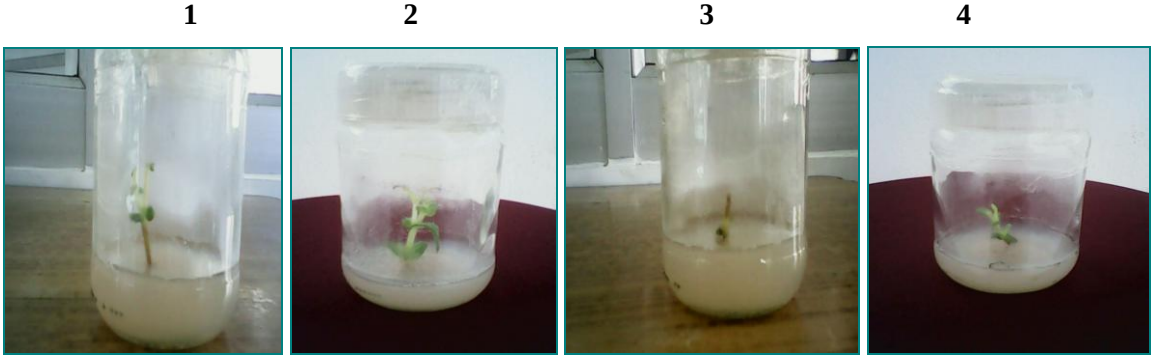

Plate3.1. *In vitro propagation of medicinal plant achyranthes aspera*

3.2. Antibacterial Activity

The medicinal properties and pharmacological actions of *Achyranthes aspera* is well used in Indian traditional medicine. Medicinal parts represent a rich source of antimicrobial agents. The plant known to contain various active principles of the rapeutic value and to possess biological activity against a number of diseases. All the extract of *Achyranthes aspera* inhibited almost all the test organism at concentrations of 25µl, 50µl, 75µl and 100 µl, it shows very high activity towards *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtillis* species at the concentration of 100 µl and the aqueous extracts *Achyranthes aspera* shows very less activity towards *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* which is shown in (Table: 1).

Table1. *Antibacterial activity of achyranthes aspera Zone of inhibition (mm)*

MICROORGANISM	AQUEOUS 100µl	CHLOROFORM 100µl	ETHANOL 100µl	METHANOL 100µl
Escherichia coli	14	40	31	32
Pseudomonas aeruginosa	6	22	40	27
Staphylococcus aureus	-	45	24	8
Bacillus subtilis	20	32	23	30

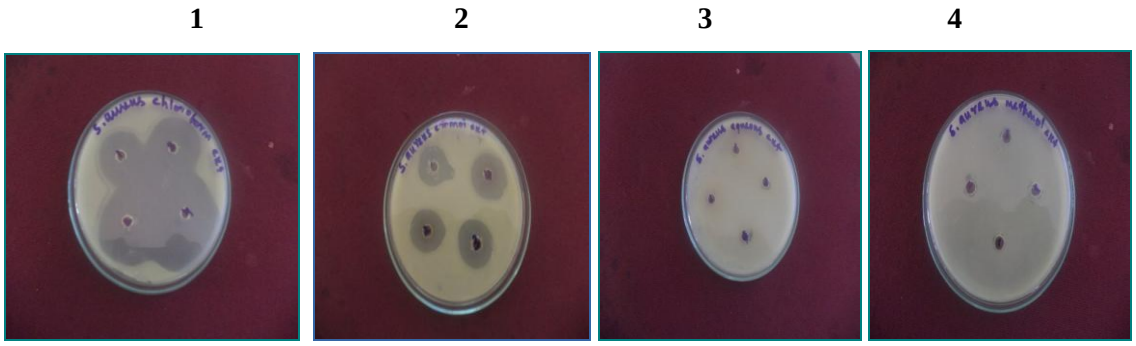

Plate3.2.1. *Antibacterial activity of various achyranthes aspera against staphylococcus aureus*

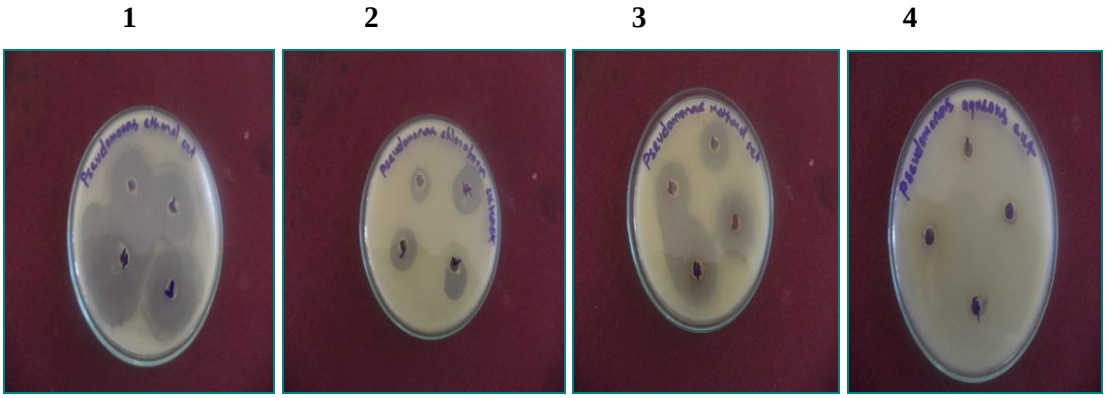

Plate3.2.2. *Antibacterial activity of various extracts of achyranthes aspera against pseudomonas aeruginosa*

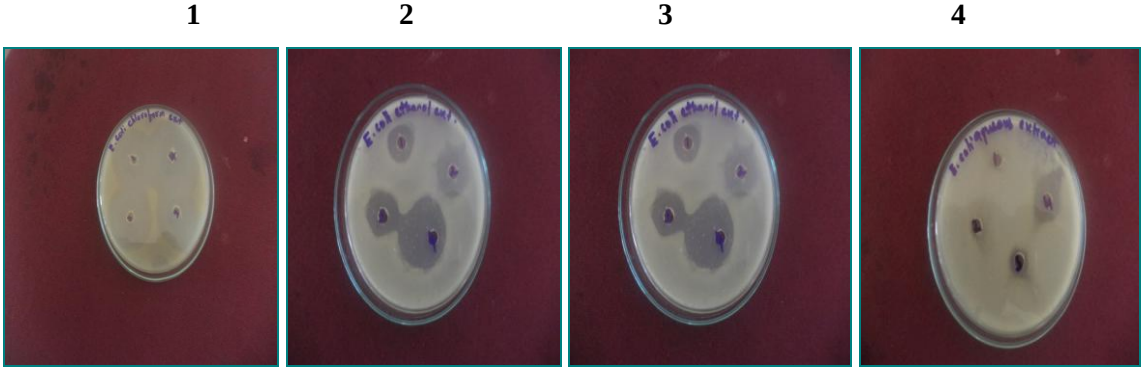

Plate3.2.3. *Antibacterial activity of various extracts of achyranthes aspera against escherichia coli*

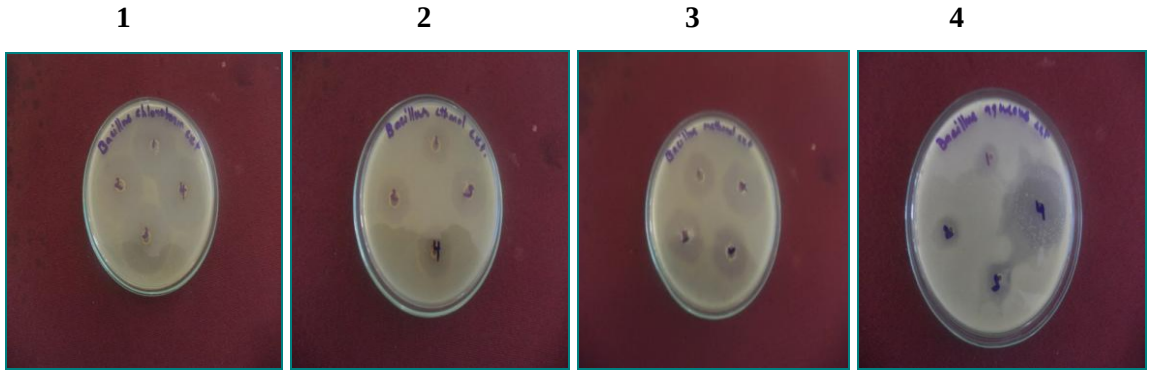

Plate3.2.4. *Antibacterial activity of various extracts of achyranthes aspera against bacillus subtilis*

3.3. Phytochemical Analysis

3.3.1. Qualitative Screening

The phytochemical analysis of extract of *Achyranthes aspera* was done and the phytochemicals like Carbohydrates, proteins and amino acids and steroids are present, which is showed in the (Table 2). Saponins, Gums and Mucilages, Phlobo-tannins and Terpenoids are absent.[1] This proves that the extract of *Achyranthus aspera* contained bioactive compounds which are responsible for its therapeutic activity.[2]

Table2. *Phytochemical analysis of achyranthes aspera*

S.NO	TESTS	LEAF EXTRACT
1	Alkaloids	present
2	Carbohydrates	Present
3	Saponins	Absent
4	Proteins and amino acids	Present
5	Phenolic compounds and tannins	Absent
6	Gums and Mucilages	Absent
7	Steroids	Present
8	Flavanoids	present

3.4. DNA Isolation from Plant Tissue

The search for a more efficient means of extracting DNA of both higher quality and yield has lead to the development of a protocols, however the fundamentals of DNA extraction remains the same. DNA must be purified from cellular material in a manner that prevents degradation. Because of this, even crude extraction procedures can still be adopted to prepare a sufficient amount of DNA to allow for multiple end uses. DNA extraction from plant tissue can vary depending on the materials used. This method has been shown to given intact genomic DNA from plant tissue.

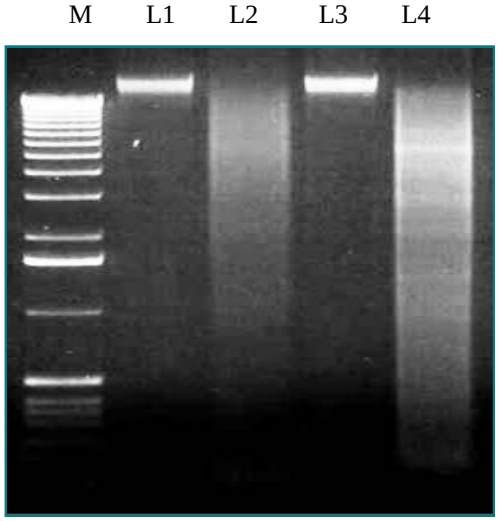

Plate4. *DNA isolation of the medicinal plant achyranthes aspera*

M - 1kb ladder

L1, l3 – sample, DNA fragments extracted from *achyranthes aspera*

To check the quality of the extracted DNA, a sample is run on an agarose gel, stained with ethidium bromide, and visualized under UV light. To confirm the DNA quality, presence of a highly resolved high molecular weight band indicates good DNA, presence of a smeared band indicates DNA degradation.

3.5. Restriction Fragment Length Polymorphism

RFLP analysis was performed for the same plant samples subjected to different DNA isolation by CTAB method. The band patterns were compared. Then the digested band were separated on 1.5% agarose gel concentration, Ethidium Bromide (0.5µl/ml), observed under UV light, and photographed.

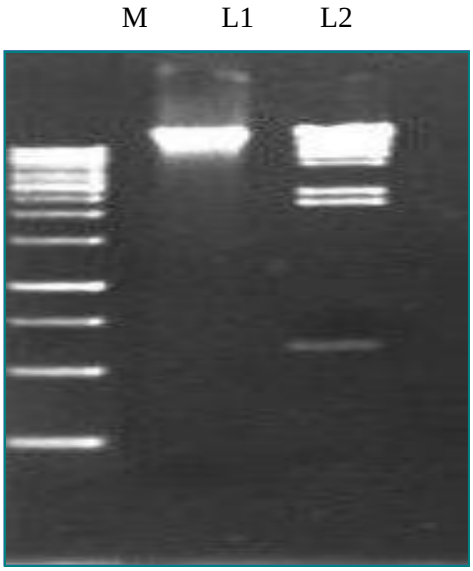

Plate5. *Rflp analysis of medicinal plant achyranthes aspera*

M – 1kb DNA ladder

L1 l2 – sample, restricted DNA fragments from achyranthes aspera

3.6. Fourier Transform Infrared Spectroscopy (FTIR)

Functional groups of *Achyranthus aspera* was analysed by FT-IR. The functional groups present in the extracts include Alcohol, Alkanes, Acid chlorides, Alkyl halides and Alkenes.

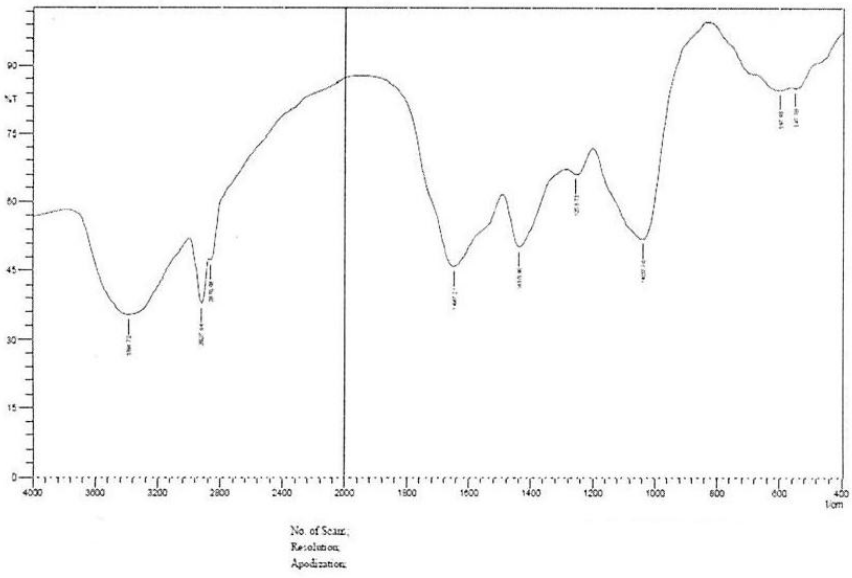

Plate6. *Ft-ir analysis of the medicinal plant achyranthes aspera*

Table3. *Ftir analysis of achyranthes aspera*

S.NO	Wave Number (cm^{-1})	Functional group
1	3394.72	Alcohol
2	2927.94	Alkanes
3	2870.08	Alkanes
4	1647.21	Alkanes
5	1438.90	Alkanes
6	1253.73	Alcohol
7	1037.70	Alcohol
8	597.93	Acid Chloride
9	547.78	Alkyl Halides

4. DISCUSSION

The importance of plants is known to us well. The plant kingdom is a treasure house of potential drugs and in the recent years has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs, antimicrobial drugs [14], antihepatotoxic compounds. According to World Health Organisation (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency (Goldberg *et al*., 2006).

The methanolic extracts of leaves of *Achyranthes aspera* has shown different activities against 22 microorganisms [16]. *Achyranthes aspera* shows antiviral activity against Papaya viruses. In addition to these *Achyranthes aspera* shows various biological activities.

Khan [15] reported that the ethanol and chloroform extracts of seeds of *Achyranthes aspera* shows mild to moderate antibacterial activity against *B. subtilis, E. coli* and *P.aeruginosa.* The various extracts of the leaves and callus of the plant also shows antimicrobial activity. Saravanan *et al*., [17] reported the solvent leaf extracts were tested for antibacterial and antifungal activities against *E. coli, P. aeruginosa, P. vulgaris, S. aureus, Klebsiella* species. Misra [18] reported 17-pentatriacontanol as a chief constituent isolated from essential oil of the shoots of plant, the oil shows antifungal activity against *Asperigillus carneus.*

The present investigation proved that all the extract of *Achyranthes aspera* inhibited almost all the test organism at concentrations of 25µl, 50µl, 75µl and 100 µl it shows very high activity towards *Staphylococcus aureus*, *Escherichia coli* and Bacillus *subtillis* species at the concentration of 100 µl and the aqueous extracts *Achyranthes aspera* shows very less activity towards *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa.*

Although all parts of the plant have demonstrated active antibacterial activity, none have shown activity against fungi.

MS medium supplemented with various concentration of BAP and Kin stimulated the shootlets formation with varied percentage [19, 20].

5. SUMMARY AND CONCLUSION

The hypothesis of obtaining plant based medicine is beneficial to human health based on active profile exposed through in vitro assays. There is a tremendous need for novel antimicrobial agents from different sources. Medicinal plants represent a rich source of antimicrobial and antioxidant agents. Antimicrobial compound is a substance that kills or inhibits the growth of microbe.

Achyranthes aspera being a very important medicinal plant has the quality of attention with its medicinal values. Conservation and propagation of all medicinal plant is a very important task to be performed in today's world for the conservation of our floral heritage and useful products from them. *Achyranthes aspera* are used externally for healing of wounds and internally for treatment of peptic ulcer. It is used for treatment of jaundice, kidney (stone), leprosy, piles, pneumonia and rheumatism.

In the present study, in vitro regeneration, antimicrobial activity, phytochemical analysis, mass multiplication was carried out. The plant was found to possess antibacterial activity against some of the pathogens. Bioactive compounds like alkaloids, carbohydrates, proteins and amino acids, steroids were identified in the extract of the plant.

Further investigation on the isolation of bioactive components from the plant would help to increase its potential to use the plant as the plant as the source of new drugs. This study also encourages cultivation of the highly valuable plant in large scale to increase the economic status of the cultivation in the country.

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