Invitro Anti-Bacterial Activity of Leaf Extract of *Eichhornia Crassipes (Mart.)* Against Significance of *Streptococcus Pneumoniae*

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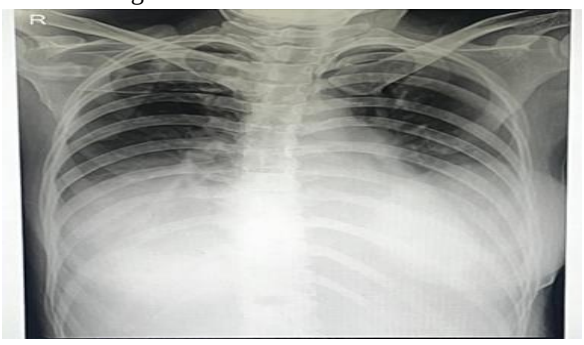
Abstract— Medicinal plants play an important role nowadays by taking part in wound healing and to treat pathological conditions. Medicinal plants serve as resource for ingredients in pharmaceutical drug manufacturing. Some herbs used as nutrition, food, perfume, natural dye, pest control. Ayurveda and other Indian literature have mentioned use of herbs. Microorganisms like bacteria, fungi, protozoa, virus, etc. cause various ailments in humans. The disease caused by these microorganisms are treated using antimicrobials. The present study is about antibacterial effect of leaf extract of water hyacinth (Eichhormia crassipes) against Streptococcus pneumonia by using ethanolic extract. Pneumonia is a microbial infection caused by Streptococcal pneumonia, it can affect lungs and cause sepsis by entering into bloodstream. The plant leaf extract was prepared using deionized water as solvent by Ultrasonic extraction method. The green color extracted product is filtered and placed in refrigerator for contamination free. The microorganism used is Streptococcus pneumonia and the culture medium is made of brain heart infusion broth (pH-7.6), incubated and turbidity adjusted to McFarland standard. The antibacterial activity was carried out by Agar well diffusion method, this leaf extract activity is compared with Teicoplanin potent antimicrobial agent. The antibacterial activity of water hyacinth is indicated by formation of zone of inhibition, it indicates diffusion of compounds into wall of microorganisms. These leaf extract exhibit broad spectrum of antibacterial activity.

Indexed Terms— Pneumonia, Antimicrobials, Antibacterial., Agar well diffusion method, Ultrasonic extraction, Zone of inhibition.

I. INTRODUCTION

The promotion and use of medicinal plants complement all current illness preventive measures and serve critical roles in disease prevention. Every day, more studies are being done and more herbal medicine is being used to cure illnesses. Due to the availability of natural chemicals, medicinal plants are a key source of molecules with therapeutic qualities. The rare and organic medicinal herbs are used to treat a variety of illnesses and generate money. The current study focuses on the understanding of plant medicinal uses and scientific research to support such uses, as well as the role, contributions, and utility of medicinal plants in treating important diseases for public health [1]. The traditional medicine practitioners provide extremely powerful recipes for treating common illnesses like diarrhoea, constipation, hypertension, low sperm count, dysentery, weak penile erection, piles, coated tongue, menstrual problems, bronchial asthma, leucorrhoea and fevers. Although the use of herbal medicine has significantly increased over the past 20 years, there is still a dearth of research data in this area [2][3]. Herbal trade is a $100 billion industry worldwide each year. The annual medicinal plant trade between India and China is between two and five billion dollars, whereas it exceeds one billion dollars in Germany [4]. Microbes are extremely minute living entities that are all around us and are invisible to the unaided eye. They are aquatic, terrestrial, and avian organisms. They comprise bacteria, viruses, fungus, protozoa, and more. Viruses are acellular organisms that are made up of proteins and either DNA or RNA for their genetic material, but never both [5]. AIDS was caused by the Human Immunodeficiency Virus (HIV) in 1981. A fungus is a eukaryote. The surface of the body and internal organs like the sinus are both impacted by black fungus. Microscopic unicellular eukaryotes known as protozoa have a very complicated internal structure and engage in intricate metabolic processes. Malaria is brought on by Plasmodium falciparum. Bacteria are found everywhere. They are crucial to preserving the ecosystem in which we live. Numerous diseases can be brought on by certain germs [6]. Infections with bacteria have a significant impact on public health, and

this is because of the numerous categories in which bacteria are categorised. For instance, illnesses may be categorised as being brought on by gram-positive or gram-negative bacteria. Humans can contract bacteria via the air, water, food, or living things. Gram-positive infections include Pneumococcal infections, Staphylococcal infections, etc., while Gram-negative infections include Cholera, Escherichia coli infections, etc. [7][8]. The bacteria that cause pneumococcal illness, Streptococcus pneumoniae, can target several bodily parts. These bacteria can cause meningitis when they enter the brain's protective layer, pneumonia when they enter the lungs, sepsis when they enter the bloodstream, and pneumonia when they enter the bloodstream. These severe illnesses can be fatal and frequently necessitate hospitalisation. In addition, the bacteria might result in sinusitis and middle ear infections (otitis media). Pneumococcus, also known as streptococcus pneumoniae, is a gram-positive, extracellular, facultative anaerobic bacteria with more than 100 recognised serotypes that is lancet-shaped. Louis Pasteur initially identified S. pneumonia in 1881 from the saliva of a rabid patient. S. pneumoniae was thought to be responsible for 95% of pneumonia cases in the days before antibiotics [9]. However, S. pneumoniae is currently responsible for up to 15% of pneumonia infections in the US and 27% of cases globally. Only 20% to 25% of all pneumonia cases caused by S. pneumonia have positive blood cultures, making the diagnosis difficult for the clinician. The upper respiratory tract (URT) mucosa is colonised by Streptococcus pneumoniae [10]. Both transmission to other people and invasive disease in the carrier are dependent on this carriage. S. pneumoniae can be shed by carriers in nasal secretions, which allows the bacterium to spread [11]. By aspiration, germs can move to other body parts and cause diseases like sepsis and meningitis in the brain and other organs.



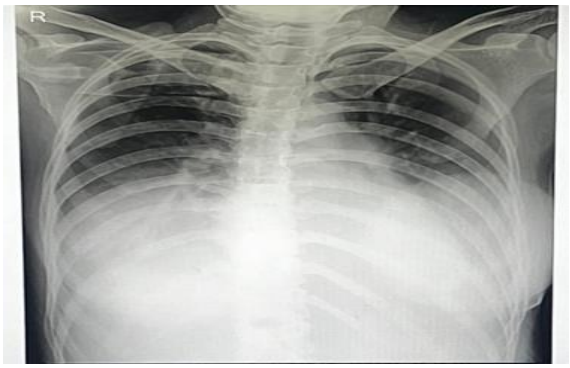


Figure 1: X Ray report of normal and pneumonia patient.

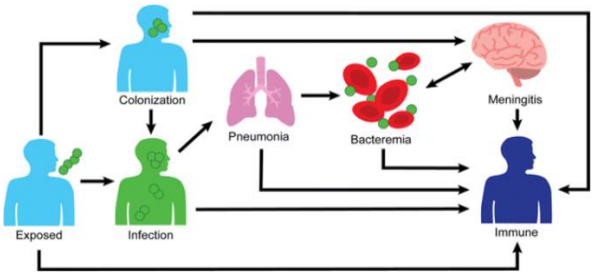


Figure 2: Affection pattern of pneumonia

There are currently four vaccines available in the most of countries (PCV13, PCV15, PCV20, and PPSV23) that help protect against a number of bacteria that cause pneumonia and other pneumococcal disease [12]. Infants, pre-schoolers, and school-aged children with suspected bacterial pneumonia may be treated with amoxicillin. Children with suspected atypical pneumonia can be treated with macrolides. Healthy adults under 65 years with pneumonia are typically treated with a combination of amoxicillin plus a macrolide like Zithromax (azithromycin) or sometimes a tetracycline like Vibramycin (doxycycline).

II. PLANT AND ITS DESCRIPTION

Figure3: *Eichhornia crassipes* (Mart.) Solm

A. DESCRIPTION

Eichhornia crassipes is a floating aquatic plant (Fig. 3), usually grows in shallow freshwater ponds, lakes, and rivers. It grows vigorously within 15–20 days and forms a dense, floating mat, over the surface of water bodies. The mature plant has roots, leaves, stolon, inflorescences, and fruit clusters. The root morphology is highly plastic and fibrous, having one single main root with many laterals, forming a huge root system. Because each lateral root has a root tip, E. crassipes may exploit nutrients in a low-nutrient water body, which makes the lateral roots longer and denser at low phosphorus concentrations [13][14].

B. PHYTOCHEMISTRY

The phytochemical composition of E. crassipes has been extensively explored, revealing diverse secondary metabolites, among them polyphenols (9.73%), flavonoids (10.49%), fatty acids (10.1%), alkaloids (7.4%), sterols (6.17%), and other compounds [15][16][17].

Table 1: Eicchornia crassipes and secondary metabolites

SLNO	Parts of plants	Secondary metabolites
1.	Flowers, leaves, stalks, and roots	Phosphatidylethanolamine, phosphatidylcholine, and phosphatidylglycerol
2.	From the different parts of the plant	Hetero polysaccharides such as L-galactose, L-arabinose, and D-xylose, hemicellulose, cellulose, glycolipids, and triacylglycerols.
3.	From leaves	leucine, asparagine, and glutamine

III. MATERIALS AND METHODS

Fresh leaves of *Eichhornia crassipes* are collect from the local area. All the Reagents, Solvents and Chemicals used for the study were analytical grade [18][19].

A. *Preparation of Eicchornia crassipes leaf extract*

The plant *(Eichhornia crassipes)* leaves are convincingly cleaned with deionised water to remove the debris that may contaminate the extract and finally dried over under the sun rays. The dried leaves were crushed using agate mortar and pestle. The ultrasonic extraction will be performed with 20 g of plant species (dried leaves) powder in the 150 ml of ethanol for about 72^0 c at 40 mins. After that, the green-coloured extract will be filtered and stored in the refrigerator to prevent side reactions and contamination-free [20][21][22].

B. *Selection of microorganism and preparation of media*

The bacterial isolates *Streptococcus pneumoniae* isolated from patients with Chronic Obstructive Pulmonary Disease (COPD). The bacteria were obtained, as clinical isolates. Subcultures were made monthly and stored at 4 °C.

C. *Culture preparation*

A loop full of 24 hr. surface growth on a NA slope of each bacterial isolate was transferred individually to 5ml of Brain heart infusion broth (pH 7.6) and incubated at 37 ^{0}C for 24 hr. bacterial cells were collected by centrifugation at 3000 rpm for 15 min, washed twice and resuspended in 0.1% peptone water. Turbidity was adjusted to match that of as McFarland standard (108 CFU/ml). Then 1:10 dilution of the cell suspension was performed to give an inoculums concentration of 107 (CFU/ml) [23][24].

D. *Minimum inhibitory concentration*

The minimum inhibitory concentration, or MIC, of a substance that combats bacteria is measured in mg/L (g/mL), and it is the smallest amount at which the strain being tested of an organism cannot grow in any way that can be seen [25]. There are two metods

1. Dilution methods - in agar, in a liquid medium (micromethod/ microdilution, macromethod/ macrodilution).
2. *Gradient methods (strips impregnated with a predefined concentration gradient of antibiotic).*

Agar dilution method procedure

A variety of concentrations are created by diluting the antibiotic that's to be tested with water. A suitable volume is then added to melted agar to yield plates with final antibiotic concentrations that represent a series of 2-fold dilutions. Then, 104 colony forming units (CFU) of bacteria that have been produced to a predetermined concentration are placed as a spot to each plate. The MIC of the antibiotic against many species of bacteria can be investigated with this

technique, which enables the testing of replicated spots of one bacterial type or different bacteria. A control plate without any antibiotics and bacterial spread plates showing that the bacteria included are in the right concentration range are required controls. At the temperature of 37 degrees Celsius, the dilution plates are subsequently incubated. After that, the plates are incubated for 16 to 18 hours; however, for bacterial populations that reproduce quickly, the incubation period may be shorter. If bacterial growth has taken place in the injected locations, the plates are checked after incubation to confirm this. Antibiotics are thought to have the least inhibitory concentration against a particular bacterium at the lowest concentration at which they inhibited bacterial growth [26].

E. *Antibacterial activity of test extract (in vitro) by using Agar well diffusion method*

The Ethanolic extracts of *E. crassipes* leaves were screened for antibacterial activity using by Agar well diffusion method. The overnight culture grown in broth was used for inoculation. The plant extracts to be tested were prepared in concentration 50mg/ml. Similarly, to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20-100 µL) of the standard and extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested [27].

IV. RESULTS AND DISCUSSION

A. Preliminary phytochemical study

The plant material was collected, cleaned, shade dried and extracted with ethanol using ultrasonic apparatus. The preliminary phytochemical studies are used to find out the phytoconstituents of the given of plant material, and used to judge the authenticity of the plants as well as to distinguish the plant material from the adulterants or allied species.

Table 2: Organoleptic characters of leaf powder of Eichhornia crassipes

S. No	Parameters	Raw
1	Appearance	Powder
2	Touch	Coarse
3	Colour	Green
4	Taste	Bitter
5	Odour	Pleasant

The preliminary phytochemical study of ethanolic extract of *(Eichhornia crassipes)* was performed and results showed the presence of alkaloids, glycosides, flavonoids, steroids, tannin, phenols, carbohydrate, protein as shown in Table 3.

Table 3 Detection of phytoconstituents in the ethanol extract of leaves of *(Eichhornia crassipes)*

S. No	Tests	Presence or Absence
1	Alkaloid	+
2	Gums and mucilage	-
3	Glycosides	+
4	Flavonoid	+
5	Phenol	+
6	Steroid	+
7	Sterols	+
8	Tannin	+
9	Terpenoid	-
10	Carbohydrate	+
11	Protein	-

Note: + indicates present; - indicates absent

Phytoconstituents in the ethanol extract of leaves of *(Eichhornia crassipes)*

Physicochemical constituents such as ash value, extractive values, loss on drying and pH of leaves of *(Eichhornia crassipes)* were shown in table

Table 4: Physicochemical values of Ethanolic extract of *Eichhornia crassipes* leaves

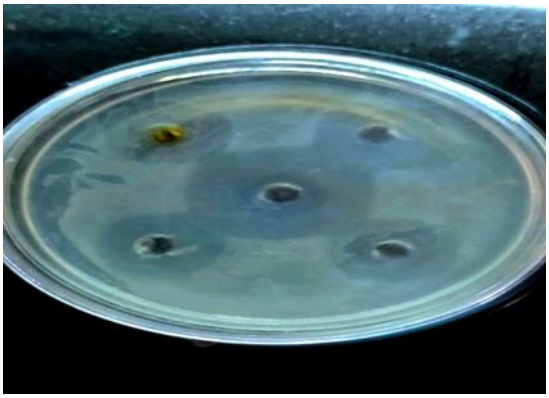
S. No	Parameters	Results
1	Total ash	19.9% ± 0.27
2	Water soluble ash	10.19% ± 0.022
3	Acid insoluble ash	4.17% ± 0.024
4	Sulphated ash	11.45% ± 0.023
7	Chloroform soluble extractive	4.09% ± 0.059
8	Acetone soluble extractive	5.6% ± 0.25
9	Ethanol soluble extractive	17.41% ± 0.024
10	Water soluble extractive	7.14% ± 0.049
11	Loss on drying	7.56 % ± 0.062
12	pH (1 and 10% w/v)	6.30±0.002

V. PHARMACOLOGICAL ACTIVITY

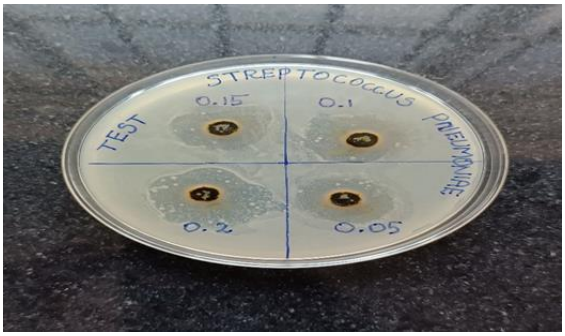
The antibacterial activity of ethanolic extract of *Eichhornia crassipes* against *Streptococcus pneumoniae* were carried out by Agar well diffusion method. In this study, ethanolic extract of *E. crassipes*, compared with antibiotic Teicoplanin, exhibited potential as antimicrobial agent. A clear zone of growth inhibition was found around the wall because of diffusion of compounds. The diameter of the inhibition zone differed according to the relative susceptibility of the test microorganisms to a particular antimicrobial agent. In general, leaves extracts exhibited broad spectrum of antibacterial activity compared with antibiotic [28].

Table 5: Ethanolic extract of E. crassipes compared with antibiotic Teicoplanin

S. No	Concentration (µg/mL)	Zone of inhibition (Diameter in mm)	
		Ethanolic extract	Teicoplanin
1	50	6.42	6.42
2	100	7.92	11.1
3	150	9.5	14.3
4	200	11.1	17.5



a) Control



b) Test

Figure4: Antibacterial activity of Ethanolic extract

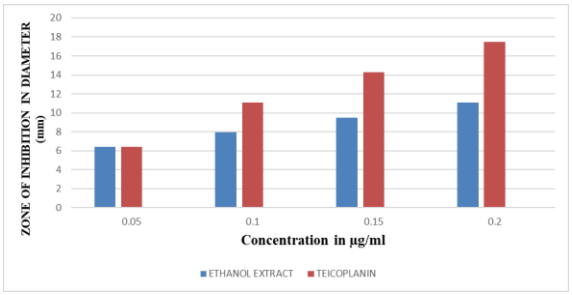


Figure 5: Antibacterial activity of *E. crassipes* against Streptococcus pneumoniae

This image indicated that the maximum antibacterial activity was found by the ethanolic extract against *Streptococcus pneumoniae.*

VI.CONCLUSION

The ethanolic extract of water hyacinth *(Eichhornia crassipes)*, which is often considered as a weed, has been recently studied by many researchers for its phytochemical and pharmaceutical applications. This study can be concluded that the leaf extracts of *Eichhornia crassipes* showed significant antibacterial activity against *Streptococcus pneumoniae*. The present study showed that the devastating aquatic weed may be useful for developing alternative compounds to treat infectious diseases caused by pneumoniae.

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