

EVALUATION OF POTENTIAL ANTIBACTERIAL ACTIVITY OF *ACHYRANTHES ASPERA* L

Mehwish Afridi¹, Zinia Tariq¹, Tahir Aqeel Malik¹, Khurram Rehman², Asad Farooq², Muhammad Akhlaq², Muhammad Zeeshan Danish³, Muhammad Imran⁴, Kamran Ahmad Khan²

¹School of Pharmacy, University of Lahore, Islamabad, Pakistan

²Faculty of Pharmacy, Gomal University, D.I.Khan, Pakistan

³University College of Pharmacy, Punjab University, Lahore, Punjab, Lahore, Pakistan

⁴Department of Pharmacy, Comsats Institute of Information Technology, Abbottabad, Pakistan

ABSTRACT

Background: Medicinal plants have been a source of treating fungal and bacterial infections since ancient times. According to WHO 80% of world population depends upon traditional medicine for treating infections and other ailments. New research has been carried out to discover plants of antibacterial origin but due to some reason some plants are not given patronage and are referred to as forgotten plants. It is need of the day to evaluate forgotten plants for their antibacterial activity.

Material & Methods: *Achyranthes Aspera* extracts were screened for their antibacterial potential. The extracts of *Achyranthes aspera* L were tested against two bacterial strains viz. *Escherichia coli* ATCC 15224 and *Staphylococcus aureus* ATCC 6538 using disc diffusion method. For checking the antibacterial activity of plant extract agar well-diffusion method was used.

Results: Among three extracts the toluene extract was most active against the test strains *Staphylococcus aureus* and *E. coli* with zone of inhibition of 18.5 mm and 12.5 mm when compared with standard Cefixime 22 mm and 23.5 mm respectively.

Conclusion: Comparing the three types of extracts, toluene extract of *Achyranthes aspera* L was found to be the most effective for antibacterial activity especially against Gram positive bacteria as compared to Gram negative bacteria.

KEY WORDS: *Achyranthes aspera* L; Anti-bacterial Activity; *Escherichia coli*; *Staphylococcus aureus*; Zone of Inhibition.

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INTRODUCTION

Blind use of antibiotics without knowing the etiology of infection and random selection of antibiotics for clinical infections have threatened health by giving rise to multiple drug resistant human pathogenic microorganisms. Antibiotic resistance develops due to the dynamics such as the interaction between antibiotic and microorganism, selection of antibiotic, environmental issues and most importantly noncompliance. In case of bacteriostatic antibiotics

noncompliance is the major cause for developing resistance.¹

In the past decade various organizations such as Infectious Disease society of America, the Centers for Disease Control and Prevention, WHO and World Economic Forum has made several reports, carried out conferences and debates on this problem. In April 2014, WHO affirmed that we are about to enter into post-antibiotic era in which minor infections can kill lots of people mainly due to resistance problem. In June 2014, the Great Britain has offered £10 million prize to the individual, firm or organizations that gives best solution to this problem.² So scientists in industry side are working hard, and similarly others are trying to discover novel antibiotic from natural sources that would be cost effective approach towards current issue.³

Corresponding Author:

Dr. Muhammad Akhlaq

Chairman

Department of Pharmacognosy

Faculty of Pharmacy, Gomal University

D.I.Khan, Pakistan

E-mail: akhlaqhayat@hotmail.com

Infectious diseases have various modes of transmission. They may be transmitted through respiratory airways, sexually transmitted diseases (STDs) or through fecal oral route via food and water. Variety of food borne diseases are still a serious problem in developing as well as developed countries. *Salmonella typhae*, *listeria monocytogenes*, *Eschersia coli* and *Staphylococcus aureus* are often stated as main etiological reasons for food borne diseases. These mentioned microorganisms are an alarm for health currently but, 20 years back they were not known to cause food borne diseases.⁴

Eat leeks in March and wild garlic in May, all the year after the physicians may play; Traditional Welsh rhyme (230 BC). In the prehistoric era medicinal plants were the only source of treatment of various diseases and even today the majority of rural inhabitants merely depend on plants for health related issues. Developing medicine by using the indigenous knowledge of plants has a variety of benefits for treatment of human ailments, due to the fact that plants are relatively economic and are easily available in bulk.⁵ Medicinal plants exhibit anti-microbial, anti-cancer, antioxidant, analgesic and anti-inflammatory properties. The essential oils of medicinal plants rather than their extracts have been useful in respiratory tract infections, UTIs, gastrointestinal and biliary system as well as skin infections. *Staphylococcus Aureus* gram positive bacteria are responsible for large number of diseases including skin infections, cellulitis, endocarditis and food poisoning. It is still a major causative agent for hospital acquired infections. Gram negative bacteria *E. coli* is part of normal flora of intestine and its involvement in lower RTIs, gall bladder inflammation and septicemia can't be denied. Current study was carried out to evaluate antimicrobial potential of *Achyranthes Aspera* leaves extract.

Achyranthes Aspera which is commonly known as latjeera (Hindi) and Rough Chaff tree (English). It belongs to the family Amaranthaceae It is an erect or procumbent, annual or perennial herb, 1-2m in height, often with a woody base, commonly found as a weed of waysides, on roadsides. Although it has many medicinal properties, it is particularly used spermicidal, antipyretic and as a cardiovascular agent.⁶

The chloroform, toluene and water extracts of *Achyranthus Aspera* I contain alkaloids saponins, carbohydrates, Flavonoids, phenols, tannins, terpenoids and sterol; various phytochemicals responsible for various reported biological activities of this very plant.⁷ A study reported that the ethyl acetate extracts

of *A. aspera* shows antiparasitic activity (dried leaf, flower and seed extract) against the larvae of cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini, 1887) (Acari: Ixodidae), sheep internal parasite *Paramphistomum cervi*.⁸ In another study carried out in year 2002 concluded that the methanolic extracts of leaves, alkaloid, nonalkaloid and saponin fractions shows cancer chemo preventive action on Epstein-Barr virus early antigen activation induced by tumor promoter 12-O-tetradecanoylphorbol-13-acetate in Raji cells.⁹ The methanolic extract of aerial parts of *Achyranthes aspera* showed hepatoprotective activity in rifampicin treated albino rats. A significant dose dependent decrease in the levels of various hepatic enzymes like SGPT, SGOT, ALKP, and total bilirubin was observed.¹⁰ The alcoholic extracts of leaves and seeds show anti-inflammatory activity in rats using carrageenan-induced paw edema method and formalin model.¹¹

MATERIAL AND METHODS

The aerial part of plant i.e. leaves were collected and were shade dried for few weeks after washing properly. Fully dried leaves were ground to powder and passed through sieve 60-mesh to get uniform sized particles. Finely ground plant material was then subjected to extraction process. 100 grams of powder was soaked in double the amount of solvent that was 200ml extraction was carried out with Toluene chloroform and water. Filtration was carried out by Whatman No: 1 filtering paper and then extracts were desiccated in open air and subject to antibacterial assay.

The extracts of *Achyranthus aspera* L were tested against two bacterial strains viz. *Escherichia coli* ATCC 15224 and *Staphylococcus auerus* ATCC 6538 using disc diffusion method. For checking the antibacterial activity of plant extract agar well-diffusion method was used. Strain of each bacterium was made by 0.75 ml broth culture comprising of 106 colony forming units (CFU) per ml. McFarland's (BaSO₄) turbidity was used as standard to compare the turbidity of test bacterium strains. Nutrient agar media was added in sterile petri plates about 14 cm above from the surface. The media was left for solidification, and then wells of 8 mm were borrowed with the help of sterile metallic borer. Sterile cotton swabs were used for swabbing of plates with a particular test strain to ensure the growth of that particular strain on the plate. Antibiotic (Cefixime 1mg/ml) was used as positive control. The plates were finally incubated for 24hrs at 37°C. After the specified time, diameters of zones of inhibition were measured in millimeters (mm) to determine the activity of test samples

against different strains.¹¹ Results were interpreted on the basis of diameter of zone of inhibition; zone of inhibition more than 20mm was regarded as the most active, 18-20mm; significantly active, 15-17mm; good activity.

RESULTS

Table 1 shows antibacterial activities of extracts *Achyranthes aspera* against S.A and E. coli when compared with the Cefixime. The toluene extract showed maximum antibacterial activity against S.A among all the three extracts tested. It showed a zone of inhibition of 18.5mm when compared with standard 22 mm. The activity was marked significant. Some activity against E. coli was also observed which was non-significant. On the other hand it was inactive against E. coli. Secondly chloroform extract also showed good activity i.e. 15 mm zone of inhibition against S.A when compared with standard 22. Water extract was active in none of the tested strains. In our study the toluene extract showed maximum antibacterial activity against the tested strains. Toluene is the best solvent for the isolation of compounds responsible for antibacterial activity of plant

Table 1: Zone of inhibition for gram positive and negative bacteria using various extracts.

Formulation	S. aureus (gram +ve)	E. coli (gram -ve)
Cefixime	22 mm	23.5 mm
Toluene extract	18.5 mm	12.5 mm
Chloroform extract	15 mm	Nil
Water extract	Nil	Nil

extracts.¹³ The water extract showed no antimicrobial activity and our results are in agreement a previous study carried out in 2003 in which the water extract was the least active against the tested pathogens.¹⁴

DISCUSSION

Bacterial strains were grown on Nutrient Agar medium. The pH was adjusted at 7.0 before solidification. The medium was autoclaved at 15 lb/inch² in pressures at the temperature of 121° for 20 min to ensure sterilization.

Disc diffusion method was followed to test of the antimicrobial activity of *Achyranthes aspera* L against two bacterial strains viz. *Escherichia coli* ATCC 15224 and *Staphylococcus aureus* ATCC 6538. Discs of 6 mm diameter were soaked with 10 µl of each extract (10 mg/ml) and air dried under aseptic conditions inside the laminar flow and placed on nutrient agar plates and incubated at 37° for 24 h. A 100 µl of bacterial suspension (108 cfu/ml) was used for spreading on nutrient agar plates. Only respective solvents (without extract) containing sterile blank discs were used as a negative control and (Cefixime 1mg/ml) was used as a positive control. After incubation, the antibacterial activity of *Achyranthes aspera* L was determined by measuring zone of inhibition in millimeter scale against individual studied bacteria. Each assay was carried out in triplicate.

The results of the antibacterial activity are given in Tables 1. Variable antibacterial effects of plant extracts were observed against bacterial strains and compared with the reference standard cefixime. In Toluene extract of *Achyranthes aspera* L, the zone of inhibition was 18.5 mm against (*Staphy-*

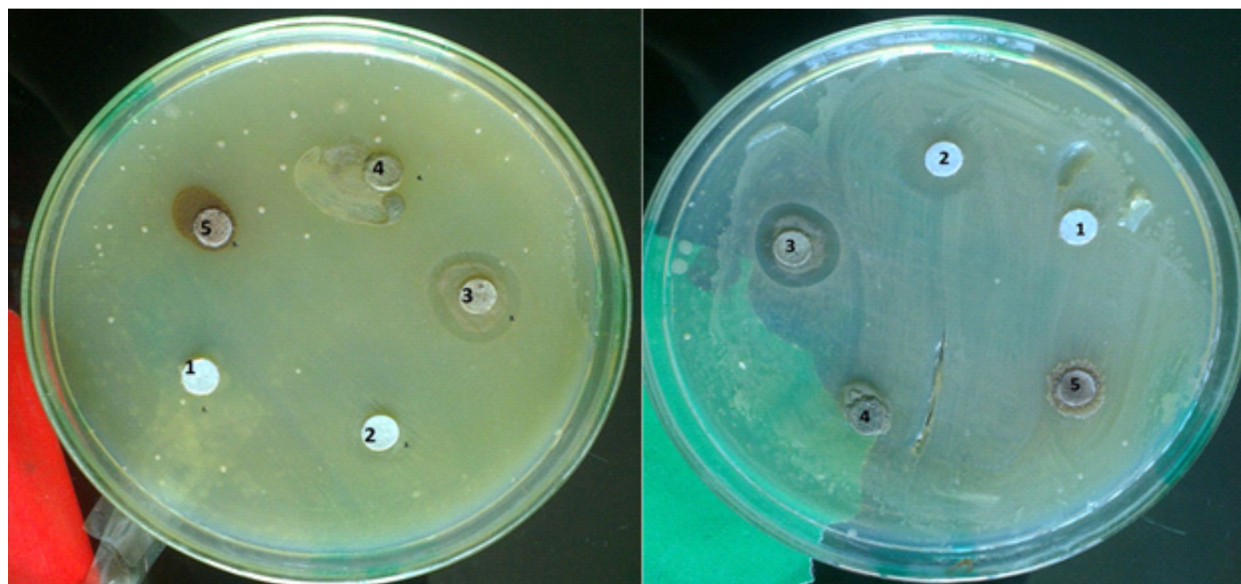


Figure 1: Zone of inhibition for gram positive and negative bacteria using various extracts.

lococcus auerus ATCC 6538) and 12.5 mm against (*Escherichia coli* ATCC 15224). On comparing with positive control it is shown that Chloroform extract of *Achyranthes aspera* L showed antibacterial property against (*Staphylococcus auerus* ATCC 6538) with zone of inhibition 15 mm. In water extract of *Achyranthes aspera* L no zone of inhibition was observed for (*Staphylococcus aureus* ATCC 6538) and (*Escherichia coli* ATCC 15224).

CONCLUSION

This data is in close agreement with that published by other researchers. Comparing among the three types of extracts, Toluene extract of *Achyranthes aspera* L was found more effective for antibacterial activity especially against Gram positive bacteria compared to gram negative bacteria. Chloroform extract was active against only gram positive bacteria. Water extract possess no antibacterial activity.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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None declared.

AUTHORS' CONTRIBUTION

Conception and Design:	MA, ZT, TAM
Data collection, analysis and interpretation:	MA, ZT, MA, ZDM
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