# STUDIES ON PHYTOCHEMICAL SCREENING ANTIOXIDANTS, ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF *ACHYRANTHUS ASPERA LINN*

**M. SELSA[a], S. SURESH KUMAR[b*], G. GOPINATH[c], M. NAGALINGAM[d], TAMIZHSELVI A**

[a] M.Sc., Student and [b,c,d,e]Assistant Professor's, Department of Biochemistry,

Indo-American College, Cheyyar-604407, Thiruvannamalai, TamilNadu, India.

*E-Mail : sureshlee6@gmail.com

***Abstract-***

The successive extracts of *Achyranthes aspera* herb parts has been investigated for *in-vitro* antimicrobial activity against *Klebsellia penumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*, by disc diffusion method. Antifungal activity against two species *Aspergillus niger* and *Fusarium oxyspporum* which has shown highest zone of inhibition concentration of 27 and 27 mm. Solvent such as Methanol was selected for extraction. The Antioxidant activity of methanolic extract of *Achyranthes aspera* by DPPH method was also carried out. Methanol extracts of *Achyranthes aspera* powder inhibited the growth of *Klebsellia penumoniae, Pseudomonas aeruginosa,* and *Bacillus subtilis* with inhibition zone diameter of 31 mm, 20 mm and 27 mm respectively. methanolic extract of *Achyranthes aspera* plant parts was found to inhibit *Escherichia coli* and *Staphylococcus aureus* with zone diameter of 30 mm and 28 mm with highest zone of inhibition concentration respectively. The antioxidant activity of *Achyranthes aspera* has shown dose dependent activity and as a good potential candidate for the role in oxidative damage. Fourier Transform Infrared Spectroscopic analysis of the leaves powder showed the presence of -OH group for phenolic compounds and UV-Visible spectroscopic analysis exhibits the presence of flavonol derivatives, carotenoids and b-cryptoxanthin epoxide as major phenolic compounds. In conclusion *A. aspera* leaves possess high antioxidant activity and can be explored as a source of natural antioxidant compounds.

**Keywords:** Phytochemicals, Pharmacological potential, *Achyranthes aspera Linn.*

## I. INTRODUCTION

An antimicrobial as an agent which is used to kill microorganisms and stop the growth. Antimicrobial medicine can grouped on basis on which microbes primarily acts against, such as, an antibacterial *i.e.*, used against bacterial growth and antifungal *i.e.* used against fungi growth. They can also be grouped on basis of their functional properties. Agents which kill microorganisms are called bacteriocidal, whereas that causes inhibition of growth are called as biostatic. The anti-bacterialial chemotherapy done by using antimicrobial medicines which are used for treatment of infection, while those antimicrobial medicines used for prevention of inflectional diseases is called anti-microbial prophylaxis. Antimicrobial agents are mainly classified as the disinfectants ("non-selective anti-microbials", bleach), are used kill wide

varieties of microorganisms and different microorganisms on non living things and the antiseptics are used for applied to livings tissues/bodies and the antibiotics destroy microorganisms inside the body. Antibacterial agents can also be further sub-divided in bactericidal agent that are used to killing bacteria, and the bacteriostatic agent that slows down and still bacteria growth. Medicinal plants are gaining much interest recently due to their special attributes as a large source of therapeutic phytochemicals that may lead to the development of novel drugs. Phytochemicals are non-nutritive plant chemicals that possess protective and disease preventing capabilities. Phytochemicals in plants include a diverse array of different chemicals such as phenolic acids, flavonoids, isoflavones, epicatechins, catechins, carotenoids, anthocyanins, alkaloids, tannins *etc.* The physiological function of phytochemicals varies from their enzymatic action to anti-oxidative nature. The anti-oxidant potential of these plant derived chemicals is due to presence of various hydroxyl groups in their chemical structures. These hydroxyl groups scavenge or quench the free radicals generated during metabolism, thus preventing the body from oxidative stress and cancers.

The pharmacological potential of these phytochemicals ranges from antimicrobial to anti-HIV nature. The superior nature of phytohemicals over allopathic drugs lies in the fat that chemicals present in plant extracts act synergistically with each other by reducing or eliminating its side effects. Knowledge of herbs using from generation to generation for hundreds of years as part of traditional. It used for medicine because of easily available and inexpensive. Phytochemicals useful as drugs in terms of broad spectrum activity for many microorganisms that leads for treating microbial diseases. Achyranthes aspera is an important medicinal plant and all parts are used in medicines, seeds and roots are the most. The present research shows updated information for pharmacological and anti-bacterial properties [1]. Nature has been an excellent source of phytochemical agents in the field of clinical microbiology [2]. Phytochemicals contain a wide range of components that is effective for bacterial diseases such as urinary tract infections. The use of plant extract for medical treatments is enjoying great popularity since 1990s. These phytochemical drugs are useful when people misuse of traditional antibiotics that cause drug resistance [3].

*Achyranthes aspera Linn* is very versatile medicinal herb found as a weed throughout India and in tropical environment. It belongs to the family Amaranthaceae and commonly known as Apamarg (in Hindi) and Rough Chaff flower in English. Its roots, seeds and flowers are mainly used for various therapeutic activities in traditional system of medicine. It an important medicinal plant used in various diseases like odontalogic, rheumatism, bronchitis, skin disease, rabies [4], fever, dysentery and diabetes. Ayurvedic system of medicine describes this plant as bitter, pungent, laxative, stomachic, carminative and useful for the treatment of vomiting, bronchitis, heart disease, piles, itching, abdominal pain, ascites, dysentery, blood disease *etc* [4-6]. Although it has many medicinal properties, it is particularly used as spermicidal [7], Antipyretic [8], abortifacient activity [9], antibacterial [10-12], antifungal [13,14], wound healing [15], anti-parasitic [16], anti-helmintic [17], and anti-hepatic activities [18]. Various kinds of medicinally important chemicals like ecdysterone, achyranthine, betaine, pentatriaontane, 6-pentatriacontanone, hexatriacontane and tritriacontane are reported to be present in different parts of Apamarga which are responsible of different pharmacological activities.

## II. MATERIAL AND METHODS

**Chemicals:**

Chemicals used in the study were 2,2-Diphenyl-1-Picrylhydrazyl Radical (DPPH), Gallic acid, Tannic Acid, Quercetin, Methanol, Ethanol, Ammonium Acetate, Folin-Ciocalteu reagent, Aluminium chloride hexahydrate ($AlCl_3.6H_2O$), Vanillin, Catechin and Sodium Carbonate anhydrous ($Na_2CO_3$). All the chemicals were purchased from Merck, India and all solvents used were of Good and Analytical grade.

**Preparation of Medium:**

Readymade dehydrated medium supplied by Hi Media was used for testing the antimicrobial activity of plant extract. The dehydrated medium was dissolved in 100 ml of distilled water and heated to boiling to dissolve the medium completely following the instructions given by manufacturer. The medium was distributed into clean glass tubes and plugged with cotton and sterilized by autoclaving at 15 lb/sq. inch pressure at $121^0$C for 20 min.

**Selection of Test Organism:**

The test organisms selected for the study are representatives of the pathogens and bacteria, which are recommended by various pharmacopoeias for antibiotic assays. The organisms used were gram positive and gram negative bacteria. These organisms were maintained on the nutrient agar and Sabaourd's agar slants. Subcultures were made on fresh medium when required. The master cultures were subcultured every month. The test organisms selected for the present study were *Klebsellia penumoniae, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Staphylococcus aureus* (Fig 3, Table 1), and fungal strains of *Aspergillus niger* and *Fusarium oxyspporum* (Fig 2, Table 1).

**Inoculum:**

Culture of test organisms was used as inoculum. Loop full of organism *Klebsellia penumoniae, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Staphylococcus aureus,* and fungal strains of *Aspergillus niger* and *Fusarium oxyspporum* was taken from the slant and transferred to a flask containing sterilized nutrient broth and allowed to grow at $37^0$C. The 18 hours culture was then platted on a nutrient agar plate to study the morphological character. The cultures from nutrient broth were centrifuged and a suspension of cells was made with the sterile saline. This culture suspension was used for further studies.

**Seeding of Culture:**

To a 12 ml of sterile nutrient agar medium in tubes, 0.2 ml of the culture suspension was added. The tubes were mixed thoroughly and poured into sterile plates and care was taken to form a uniform layer.

**Controls for the Test:**

The controls were required to confirm all the necessary nutritional conditions were suitable for the growth of microorganism and for absence of inhibitory substance in the medium. The positive controls were observed by streaking the organism on agar plates for observing morphology of colonies. Any contamination during the assay was ruled out by keeping the negative control. This was checked by adding the sterile saline and observing for growth as a contamination. The results indicated that the medium was free from contamination.

**Preparation of Plant Extracts:**

The leaves of plant *Achyranthes aspera Linn* were dried and ground to powder. 200 mg of the powder was taken in 40 ml of solvents methanol, allowed to stand for 24 hours and filtered through Whatmann filters No. 41 and collected in to tubes. The filtrates of solvent were kept for evaporative drying at $70^0$C on water bath. To the dried tubes 20 ml of dimethyl sulfoxide was

added and sonicated to dissolve the active constituents. The sterile discs of 5 mm diameter were impregnated in sample and blank / controls were placed aseptically in the nutrient agar plates. The plates were incubated at $37^0$C for 24 hours, later the plates were observed for zone of inhibition of microorganism growth as well as fungal growth. The inhibition zone was measured with vernier caliper. The results were tabulated and represented in Table 1. Methanolic extract was also plotted (Control, 100, 500 and 1000 µl/ disc) as positive control Vs Blank and been proved presence of good antimicrobial activity as well as antifungal activity of the extract.

**Phytochemical Screening:** [19,20]

The dry methanolic extract of *Achyranthes aspera* (Fig 1) were screened for the presence of various phyto-constituents / secondary metabolites which are responsible for the therapeutic values of the drug for example presence of tannins, alkaloids, glycosides, carbohydrate, tannins, proteins & amino-acids, gums, mucilage, flavours, flavonoides, saponins and steroids and Sterols *etc.,* The resulting data obtained were recorded in the table.

**Detection of Alkaloids:**

The methanolic extract is warmed with $H_2SO_4$ of 2% for 2 minutes and is filtered, then few drops of reagents was added which indicated the presence of alkaloids.

**Dragendroff's Test:**

To extract (5ml) few drops of Dragendroff's reagent added. It given formation of orange color precipitate. An orange precipitation indicated the positive test for presence of alkaloids.

**Mayer's Test:**

To the extract (5ml) few drops of Mayer's reagent was added which given formation of creamy-white colour precipitate. The creamy-white precipitation indicated the presence of alkaloids.

**Wagner's Test:**

To the extract (5ml) few drops of Wagner's reagent added which formed Reddish-brown color precipitate, reddish brown coloured precipitation indicated presence of alkaloids.

**Hager's Test:**

To the extract (3ml) few drops of Hager's reagent Picric Acid (1%) was added, formation of prominent yellow color precipitate took placed. Presence of yellow color precipitation indicated positive test for alkaloid.

**Test for Flavonoids:**

A small quantity of extract was heated with ethyl acetate (10 ml). It was done by boiling water for about 3 minutes. The mixture was filtered. Filtrates were used for following test.

**Ammonium Test:**

Filtrate was shaken with dilute ammonia solution (1ml of 1%), separation of layer was allowed by keeping it stable. A yellow color was observed at ammonium layer in test tube, these indicated the presence of flavonoids.

**Aluminium-Chloride Test:**

The filtrates were shaken with aluminum chloride solution (1ml of 1%), then observed the formation of light-yellow color, this indicated presence of Flavonoids, further diluted with NaOH and HCl added. A yellow solution turns colorless indicating positive test for presence of flavonoids.

**Test for Terpenoids:**

**Salkowski Test:**

To the chloroform (2ml) and concentrated $H_2SO_4$ (3ml) was mixed with extract and was added, formation of layer was observed. Reddish-brown color of interface is formed positive result of terpenoids.

**Test for Tannins:**

To the small quantity of extract (5ml) of 45% solution of ethanol was boiled with for 5 minutes. The mixture was cooled. Filteration was done. Filtrates were used for following test.

**Lead Sub Acetate Test:**

To the different filtrate (1ml), three drop of lead acetate solution was added. Cream gelatinous precipitation was observed which shows positive test for Tannins.

**Ferric Chloride Test:**

Each of filtrate (1ml) was diluted by using distilled water. 2 drops ferric chloride was added. Transient greenish-black color formation indicated presence for Tannins.

**Test for Sterols:**

**Liebermann-Burchard Test:**

To small amount of extract chloroform (3-5 drops), acetic anhydride and $H_2SO_4$ were added along sides of test tube to observe formation of dark red/pink color. The observation of dark red/pink colour formation indicated presence of sterols.

**Test for Proteins:**

**Ninhydrin Test:**

To the test solution (1ml) Ninhydrin solution (2%) was added. Violet color indicated presence for protein in sample of extract.

**Biuret Test:**

To the extract (3ml) few drops of sodium chloride (10%) and copper sulphate (1%) was added which gives formation of violet/purple color, and on adding alkali, becomes dark purple.

**Xanthoprotein Test:**

To the extract (3ml) few drops $HNO_3$ was added. Formation of intensely yellow color indicated the presence of proteins in the sample extract.

**Test for Carbohydrates:**

**Molisch's Test:**

To small amount of extract Molisch's reagent (5-6 drops) was added follow by addition of concentrated $H_2SO_4$ along sides of test tube. Mixture was allowed to keep for two minute. It was diluted with distilled water (5ml). The Forming of red/dull violet colour at interial phase of 2 layers indicates presence of the carbohydrates.

**Fehling's Test:**

The extract (5 ml) Fehling's reagent and Fehling's reagent B was added. Kept for boiling in water-bath. Formation of yellow/red color precipitate indicated presence of reducing carbohydrate.

**Test for Glycosides:**

The diluted Sulphuric acid (5 ml) was added to extracts in test tube. It was boiled in a water bath for 15 minutes. It was cooled and potassium hydroxides solution (20%) neautralized. mixture of equal parts of Fehling's solution A and B (10 ml) was added. It was again boiled for few minutes denser red color precipitate formed indicates presence of glycosides.

**Baljet's Test:**

To the extract (5 ml), sodium picrates (5 drops) were added. It was observed change in yellow to orange colour. It indicated the presence of glycosides.

**Keller-Killiani Test:**

To the extract (5ml), ferric chloride solutions (5-7 drops) were added. It was stirred properly by stirring, then sulphuric acid and ferric chloride solution were added forming 2 layer, brownish-red and upper layer greenish blue indicated presence for glycosides.

**Test for Saponins:**

**Foam Test:**

To the extract add distilled water and vigorous shaken until foam observed.

***In Vitro* Free Radical Scavenging Activity:**

**DPPH Radicals Scavenging Assay:**

DPPH radical (Graph 3), is a widely used method to evaluate the free radical scavenging ability of natural compounds. This assay is based on the measurement of the scavenging ability of antioxidant substances toward the stable radical. The antioxidant activity of methanolic extract of *Achyranthes aspera Linn* was measured in term of hydrogen donating or radical-scavenging ability using the stable DPPH method [21-23]. The free radical scavenging activity of *Achyranthes aspera linn* were diluted in methanol at various concentration (1.25, 2.5, 3.75, 5, 6.25 and 7.5 µg/ml), then 1 ml of each diluted extract was added to 0.5 ml of 20 mg/l DPPH methanolic solution. The mixture of extract concentration and DPPH were placed in the dark at room temperature for 30 minutes. The absorbance of the resulting solution was then read at 517 nm. The antiradical activity was expressed as $IC_{50}$ (g/ml). Ascorbic acid was used as standard. The ability to scavenge the DPPH radical was calculated according to the equation.

Where $A_0$ is the absorbance of the control at 30 minutes and $A_1$ is the absorbance of the sample at 30 minutes. All samples were analyzed in triplicates.

$$Radical\ scavengiing\ activity\ (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

## III. RESULTS AND DISCUSSION

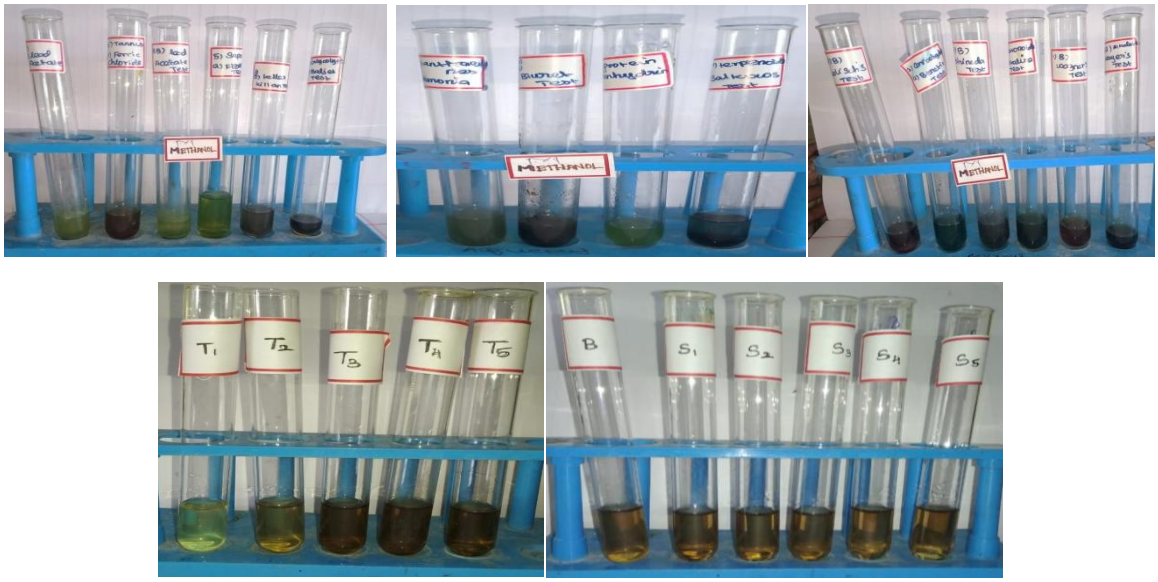



**Fig 1. Phytochemical Screening and Antioxidant Studies of Methanolic Extract of *Achyranthes aspera Linn.***

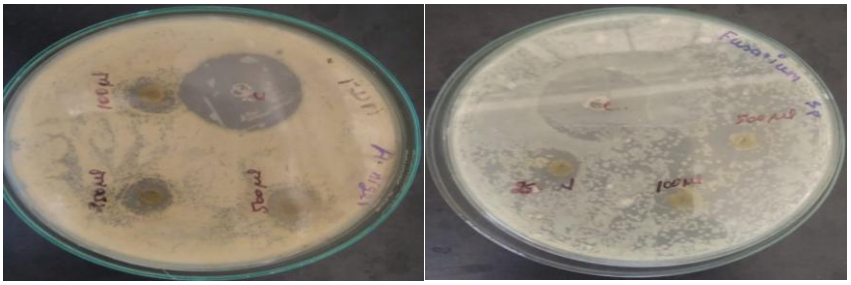


**Fig 2. Antifungal Studies of Methanolic Extract of *Achyranthes aspera Linn.***

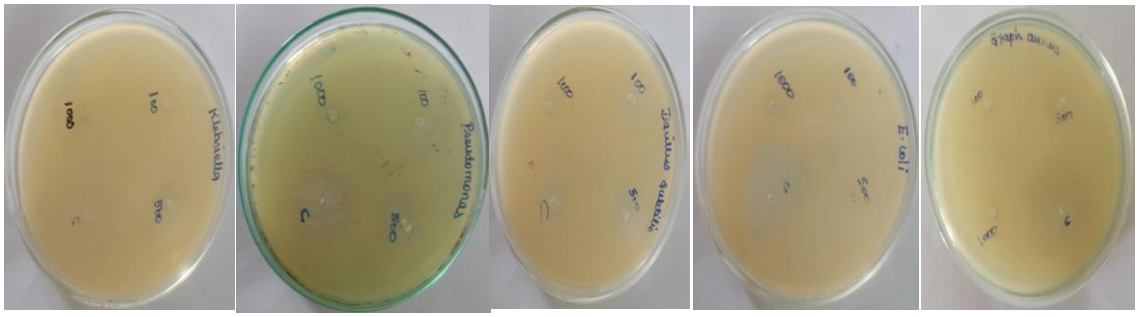
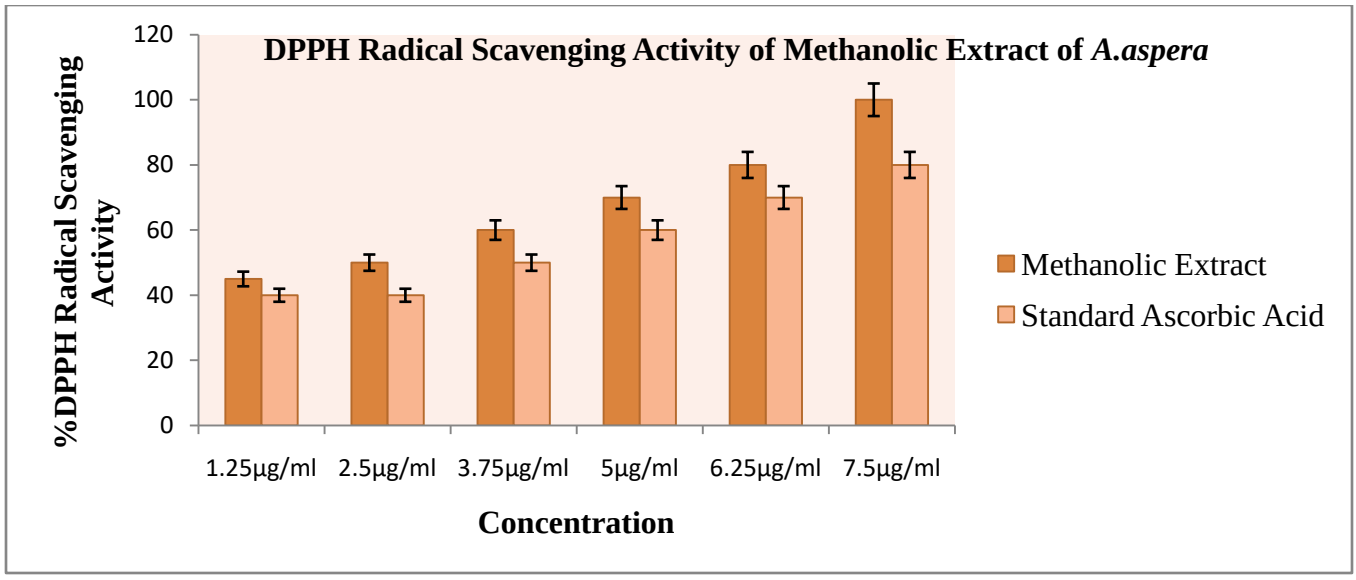


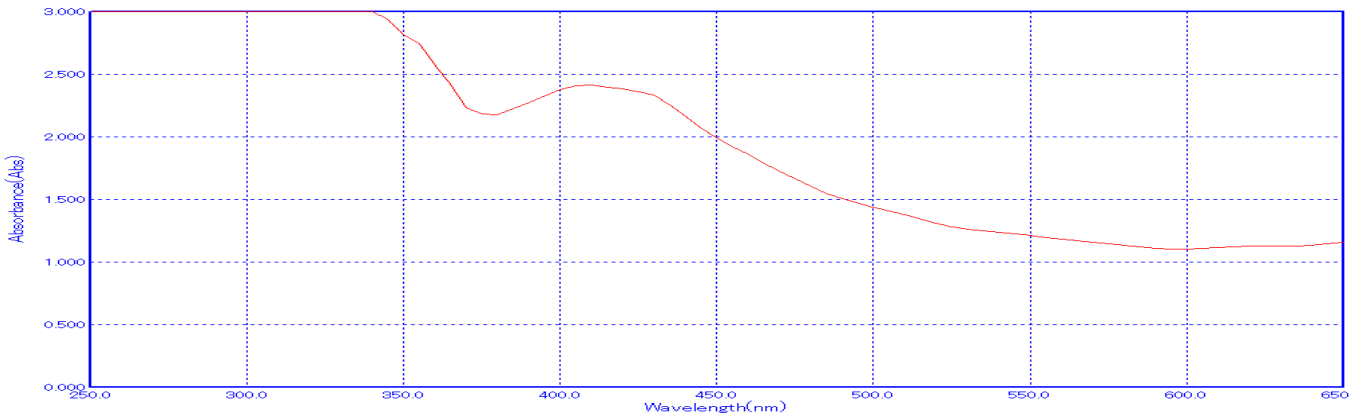
**Fig 3. Antimicrobial Studies of Methanolic Extract of *Achyranthes aspera Linn.***

**Table 1. Inhibition of growth of bacteria and fungi for fraction extract sample and zones measurement.**

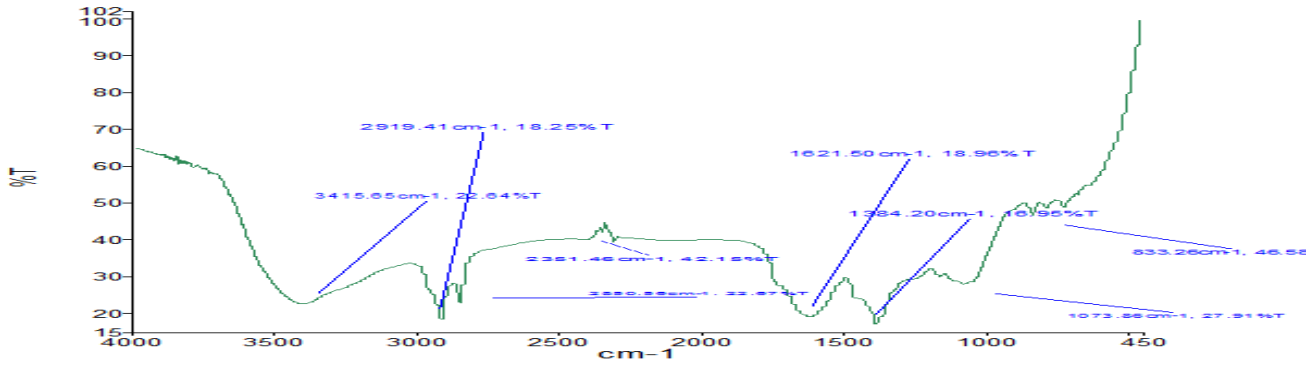
| Medicinal Plant | Target Organism | Zone of inhibition (mm) as produced in methanolic organic solvent extract of the plant | | | |
|---|---|---|---|---|---|
| | | METHANOL | | | |
| | | BACTERIA | | | |
| | | Control | 100 | 500 | 1000 |
| *Achyranthes aspera Linn* | *Klebsellia penumoniae* | 32 | 10 | 2 | 31 |
| | *Pseudomonas aeruginosa* | 20 | 16 | 20 | 11 |
| | *Bacillus subtilis* | 27 | 20 | 20 | 11 |
| | *Escherichia coli* | 30 | 14 | 16 | 10 |
| | *Staphylococcus aureus* | 28 | 10 | 17 | 11 |
| | FUNGI | | | | |
| | *Aspergillus niger* | 27 | 10 | 12 | 13 |
| | *Fusarium oxyspporum* | 27 | 10 | 12 | 14 |




**Graph 3. DPPH radical scavenging activity of the *A. aspera.* All values represent the Mean ± SD (n = 3 test).**




**Graph 4. UV spectrum of *A. aspera***




**Graph 5. FTIR spectrum of *A. aspera***

Phytochemicals is a fundamental requirement and pharmaceutical drug products. According to WHO, phytochemicals need to be standardized with safety guideline before releasing into market [24]. In this study, *Achyranthes aspera Linn* was used in our lab to screen the phytochemicals, antioxidant activity, antimicrobial and antifungal activity. The results revealed that extracts of *A. aspera* exhibited significant antimicrobial activity against the microbes as well as fungi. Extracts from leaf part of *A.aspera* appeared to be more potent and the methanolic extract produced consistent level of inhibition of bacterial growth when these results compared with the leaves of *Senna alata* which was also demonstrated significant antibacterial and antifungal activities with methanol extraction in the literatures [25]. It is interesting to note that the leaf extract of *A. aspera* could be used against multi drug resistance organisms. *A.aspera* studied for the antibacterial activity, most of them acted against pathogens. The outcome of the study encourages taking up screening the preliminary phytochemical analysis of leaf extract.

Oxidative damage to the cell results in to several physiological and neurodegenerative disorders. The oxidative damage is because of the generation of free radicals during the oxidation process [26,27]. Oxidative damage is usually countered in body by antioxidant defiance mechanism. When the amount of free radicals increases extensively high to overcome antioxidant defiance mechanism, it results in to oxidative damage to cell [28]. Oxidative damage can be controlled by the therapeutically use of antioxidant compounds. These antioxidants are naturally produced by plants and microbes to protect them from several disorders and stress conditions. The plants are a major source of antioxidant compounds and several antioxidant compounds have already reported from plants. Phenolic or polyphenols are the major phytochemical class of the plant contains the antioxidant properties. Several class of phenolic compounds are reported to be present in plants includes phenols, benzoquinones, phenolic acids, polypropenol, flavonoids, isoflavonoids phenylpropanoids, phenolics quinines, lignins, melanins, tannins *etc.,* [29]. These phenolic compounds can be isolated from plants and can be used as natural antioxidant compounds. Selection of *A. aspera L* for the study was based on its tradition use to cure several diseases and therapeutic potential reported earlier. The plant root is already reported to possess antioxidant property, so in this study we screened the antioxidant potential of the methanolic leaves extract of *A. aspera* as antimicrobial and antifungal.

DPPH radical scavenging activity was found to be $IC_{50}$ 8.957±0.279) µg/ml, where the methanolic extract were used for the estimation of antimicrobial activity and antifungal activity. The powder of *A.aspera* methanolic leaves was screened for the presence of major phytochemical groups. The results showed the presence of carbohydrates, phenolic compounds, saponins, alkaloids, oil and fats and tannins, whereas, proteins and flavonoids.

Antioxidant potential of the methanol extract was measured and expressed as % inhibition by DPPH (Graph 3) radical scavenging activity. The DPPH radical scavenging activity was found to be increasing as dose increases. *A. aspera* roots have been reported to possess DPPH radical scavenging activity with $IC_{50}$ values 241.86 µg/ml respectively [30]. Present study reports the high DPPH radical scavenging activity of *A. aspera* leaves than that of roots reported earlier. Antimicrobial activity of methanol, extract was determined by disc diffusion method. Results are reported in Table 1. The plant showed very poor antibacterial activity however, earlier studies reported the antifungal activity of the plant [31]. Further work is warranted to isolate and characterize the active principles available in the extracts of *A. aspera*. It is quite sure that such components could be useful in developing drugs [32].

## IV. CONCLUSION

The selection of *A. aspera* for the present study was based on their traditional use to treat infectious diseases (bacterial & fungal) like dysentery, skin and urinary tract infections, enteric fever and venereal diseases. *E. coli* is mainly responsible for causing urinary tract infections in humans [33]. Due to lake of appropriate health facilities, these diseases are commonly treated with local herbs. Our results against the microbes showed that *A. aspera* leaves have some potential compounds that could provide a scientific base for the treatment of stated infections by the herbal practitioners (Hakims). *A. aspera* is also used in treatment of skin and lungs diseases, since *A. niger* usually affect lungs [34], results of the present study indicates association between traditional therapeutic use of *A. Aspera* and the *in vitro* antifungal screening. These results corroborate the importance of ethnobotanical surveys for screening plants as a potential source for bioactive compounds. Phytochemical screening of *A. aspera* shows the presence of various chemical substances such a phenolic compounds, oils, saponins, flavonoids, alkaloids and tannins [35]. Flavonoids and many phenolic compounds act as antimicrobial agent against many pathogenic bacteria and fungi such as *S. aureus*, *P. aeruginosa*, *B. subtilus*, *E. coli*, *A. niger* and *F. oxyspporum* [80]. From the Graph 5 the presence of phenolic compounds was further confirmed by FT-IR analysis. The absorption band at 3415.65 $cm^{-1}$ is representative for -OH group of phenols. UV-Visible spectroscopic analysis exhibits the presence of flavonol derivatives, carotenoids and b-cryptoxanthin epoxide as major phenolic compounds as peak appeared at 400 nm (Graph 4). In light of the abovementioned facts, this preliminary study could result in discovery of novel antimicrobial drugs for the prompt and effective management of infectious diseases. It can be concluded from the present data, that the leaves of *A. aspera* posses considerable antimicrobial activity. Since methanol fraction shows promising antifungal activity against *A. niger* and *F. oxyspporum* which is a common dermatophyte therefore, further investigation is needed to isolate the specific compound and to make a safe and more effective topical dosage form. Antioxidant potential of the methanolic extract was measured by DPPH radical scavenging activity. The results are expressed as % inhibition of DPPH. Methanol extract showed high antioxidant activity. The DPPH radical scavenging activity was found to be increasing as dose increases. With the results obtained in the present study, we conclude that *A. aspera* leaves can be used as a good source for the isolation of safe and natural antioxidant compounds.

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