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Evaluation of Pharmacognostical Study and Antibacterial Activity of Leaves *Achyranthes aspera*

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ABSTRACT

The Plants are known for their diverse pharmacological activities including antibacterial activity. In the present work an attempt has been made to find out the antibacterial activity of various solvent extracts of Achyranthes aspera Linn. (Amaranthaceae). Chloroform, Petroleum ether, ethanol, methanol, and water solvent extracts of leaves of the plant were screened for anti-bacterial activity. This study concludes that the plant extracts were active against some gram-negative bacteria.

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1. INTRODUCTION

Nowadays, herbal medications stand for safety in contrast to synthetic drugs, which are seen as being hazardous to both people and the environment. A global "herbal renaissance" is taking place as herbs make a comeback. Nowadays, herbal remedies offer safety in contrast to synthetics, which are deemed hazardous to both humans and the environment. (Mukherjee, 2013) [1]

Achyranthes aspera, also known as Latjeera (Hindi) and Rough Chaff tree (English), is one of the many plants that are being examined for their therapeutic efficacies. It is a 1-2 m tall, upright or procumbent, annual or perennial herb that is frequently seen as a weed of waysides on roadsides. Despite having a wide range of therapeutic uses, it is notably effective as a spermicidal, antipyretic, and cardiovascular agent.

Chemicals such as 10-tricosanone, 10-octacosanone, and 4-tritriacontanone are present in the seeds. (Rastogi and Malhotra, 2004) [2]

The plant has historically been used to treat coughs and asthma. It helps with oedema, dropsy, piles, boils, skin eruptions, etc. It is pungent, anti-phlegmatic, antiperiodic, diuretic, purgative, and laxative. In order to treat pneumonia, crushed plant is cooked in water. A mild astringent is produced when the root is infused for intestinal issues. (Patil and Sharma, 2013) [3]

The blooming spikes or seeds are pulverised and put into a paste with water, which is applied externally to treat cutaneous illnesses and dangerous snake and reptile bites. The herb is helpful for treating rheumatism, scabies, and other skin conditions as well as liver issues. Additionally, it has calming qualities.[4]

Fig 1: Achyranthes Aspera

2. Achyranthes aspera Linn

Taxonomic classification [5]

Kingdom - Plantae

Class – Mangoliophsida

Order - Caryophyllales

Family - Amaranthaceae

Genus – Achyranthes

Species – Aspera

3. Botanical description:

Synonyms Latin - Achyranthes aspera

Sanskrit - Aghata, Apaamaarga

Hindi - Latjira, Chirchira

Tamil - Shiru-kadaladi

Telugu - Uttaraeni

Malayalam - Kadaladi

Arabian - Atkumah

4. Distributional range:[6]

It is found all throughout the tropical world. It grows as a common plant along roadside edges in numerous locations, including India.

5. MATERIALS AND METHOD

Habitat: The plant can thrive in semi-shade (light woods) or in the absence of any shade. It needs moist soil to flourish, however it favours light sandy, medium loamy, and heavy clay

soils. Everywhere it grows as a wasteland plant. From July to September, flowers bloom, and October is when the seeds ripen.[7]

Botanical description [8-9]: A little tree, Achyranthus aspera, can reach a height of 0.2 to 2.0 metres. When dry, the stem is erect, cylindrical, solid, hairy, yellowish-brown, and hollow. Simple, subsessile, somewhat acuminate, wavy-margined, ovate, petiolate, or elliptic leaves with ovate shapes and glabrous surfaces. Greenish-white, numerous, sessile, bracteate with two bracteoles, one spine-lipped, actinomorphic, and hypogynous flowers are grouped in long spikes from inflorescences. The seeds are endospermic, spherical at the base, subcylindric, and truncate at the apex. They are also brown in hue.

Preparation of samples

Young stems of Achyranthes aspera's leaves and leaflets were removed from their branches. For use in future anatomical studies, some freshly cut leaves of these plants were preserved in formaldehyde-acetic acid and alcohol (FAA). The residual bulk was shade-dried for two weeks before being ground to 60 mesh sizes and packaged individually in airtight containers for chromatographic, physicochemical, and powder microscopy research.[10]

Extraction with different solvents

The Soxhlet extraction method integrates the advantages of the reflux extraction and percolation, which utilizes the principle of reflux and siphoning to continuously extract the herb with fresh solvent. The Soxhlet extraction is an automatic continuous extraction method with high extraction efficiency that requires less time and solvent consumption than maceration or percolation. Process: Approximately 10 gram of dried powder Achyranthes aspera leaves were weighed and placed into a round bottom flask with 300 ml of the extracting solvent. The sample were extracted using soxhlet extraction method with different types of solvents including Petroleum ether, Chloroform, Ethyl acetate, Methanol and water (H2O). Process duration of the extraction used was six hours and temperature of extraction based on the boiling point of solvents. The extract from Achyranthes aspera was filtered through filter paper (Whatman No. 1) with Buchner filter under vacuum. The extract from H2O were kept in freezer at - 25°C prior to freeze dry process and organic solvent extract stored at room temperature before solvent recovery process. Then, H2O extract was freeze-dried in order to remove the solvent. However, the extract from Petroleum ether, Chloroform, Ethyl acetate and Methanol were recovered using rotary evaporator under

vacuum. The evaporation process was conducted at 45°C to minimize any possible degradation of the phytochemicals in the samples.[11]

6. Preliminary phytochemical analysis

The extract was tested for the presence of bioactive compounds by using following standard methods.

Detection of Carbohydrates

Fehling's test: 0.2gm filtrate is boiled on water bath with 0.2ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar. 44 Fehling's solution A: Copper sulphate (34.66g) is dissolved in distilled water and made up to 5s.

Detection of Proteins and Amino acid

100 mg of extracts were dissolved in water (10 ml) and then it was filtered. The filtrate was used to test the presence of proteins and amino acids.

Ninhydrin test: to the test solution added 1 ml of 0.2 % ninhydrin solution, violet colour indicate the presence of amino acids in sample.

Millon's test: Added 5 drops of millon's reagent to 1 ml of test solution and heated on awater bath for 10 min, cooled and added 1% sodium nitrite solution. Appearance of red colour confirmed the test.[12]

7. Antibacterial Activity

Tested Microorganisms. A total 4 bacteria having 2-gram negative Escherichia col, Pseudomonas aeruginosa and 2-gram positive Staphylococcus aureus and Bacillus subtilis were used to check the effectiveness of ethyl acetate extract of the Achyranthes aspera medicinal plants. [13]

8. Result and Discussion

Collection and Identification of Plant material

The plant of *Achyranthes aspera* was collected in village of Sundernagar, District Mandi, Himachal Pradesh in the month of August to September.

Assessment of quality of plant materials

Macroscopic evaluation

The leaves of *Achyranthes aspera* are simple, hairy, and shorty stalked. The leaves have two colors, i.e., yellowish below and green above, situated opposite each other on a sharp-pointed woody stem. The leaves are 8 to 10 cm long, 7 to 8 cm wide with a broad base and pointed tiplike an egg.

Figure 8.1: Leaves of the *Achyranthes aspera*

Microscopically Characters

T.S of leaf of *Achyranthes aspera* showed that the leaf is dorsiventral and hypostomatic. The lamina shows a single layered epidermis on the upper side composed of cubical cells. The upper epidermis shows mostly uni, bi and multicellular hairs. Glandular hairs are rare. The epidermisis followed by a layer of hypodermis which is usually 3-5 layers of cells thick and is interruptedat places by the palisade layer. The cells of lower epidermis are cubical in shape mostly with unitricellular trichomes. Three vascular bundles are scattered in ground tissue consisting of thinlayered parenchymatous cells. Vascular bundle consists of xylem vessels, trachids and xylem parenchyma, Phloem consists of sieve tubes, companion cells and phloem parenchyma and pericycle. The pericycle is made of 2-3 layers of thick-walled, nonlignified cells.

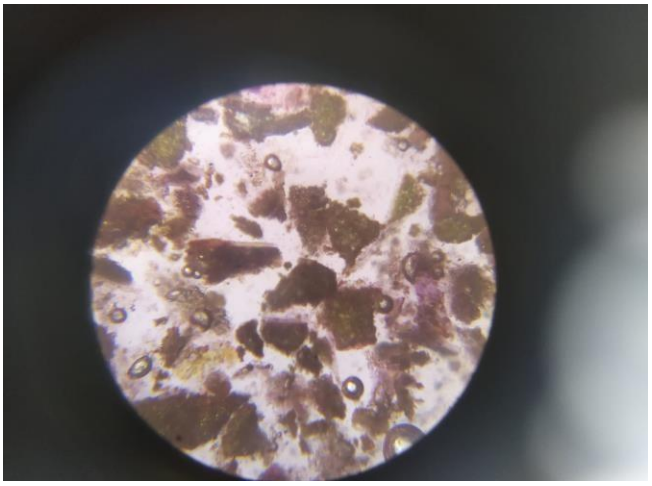


Figure 8.2: T. S. of *Achyranthes aspera* leaf.

Moisture content determination

Moisture is one of the major factors responsible for the deterioration of the drug and herbal formulation because the presence of moisture in a crude drug can lead to its deterioration due to activation of certain enzymes or due to growth of microbes.

The percentage moisture content was dried leaves of the *Achyranthes aspera* was as follows (Table 8.1):

Table 8.1: % Moisture content of leaves of the Achyranthes aspera

Plant material	**% Moisture content**
Leaves	0.50± 0.46

This study recorded moisture range of 0.3- 0.7% which is deemed to be good as water content in vegetable drugs should not be greater than 14%.

Ash values

Ash is an inorganic substance left over from the combustion of an organic material. Ignition process that is all organic substances will burn into black charcoal, with continuous heating, all organic substances (charcoal) will be burned out and ash will be obtained in the form of theremaining substances consisting of inorganic substances in the form of metal oxides. The growth process of a plant in nature requires nutrients, including those from minerals and other organic compounds. The parameter to determine the type of nutrient that is predominantly attracted to a plant is the water soluble and insoluble ash content. Water-

soluble ash content isobtained by dissolving the ashes in water and which is not dissolved filtered using ash-free filter paper (Whatman 42). This ash-free filter paper has low inorganic content and leaves only a very small amount of ash weight when burned. Filter paper and residue are resurfaced until the ash weight is constant, while the acid insoluble ash content is the total ash obtained which is dissolved into acid, which is HCl. Insoluble ash is filtered usingash-free filter paper and resumed to a constant ash weight.

Fig 8.3 Determination of ash value **Fig 8.4 Determination of acid**

Insoluble ash value, water insolubleash value and sulphated ash

Table 8.2: Total ash value, Acid-insoluble ash, Water-Insoluble ash, Sulphated ash of leaves ofthe *Achyranthes aspera*.

Sr. No.	Plant material	Total ash value (%w/w)	Acid-insoluble ash (%w/w)	Water-In soluble ash (%w/w)	Sulphated ash (%w/w)
1	Leaves	0.65±0.21	0.57±0.18	0.72±0.11	1.18±0.13

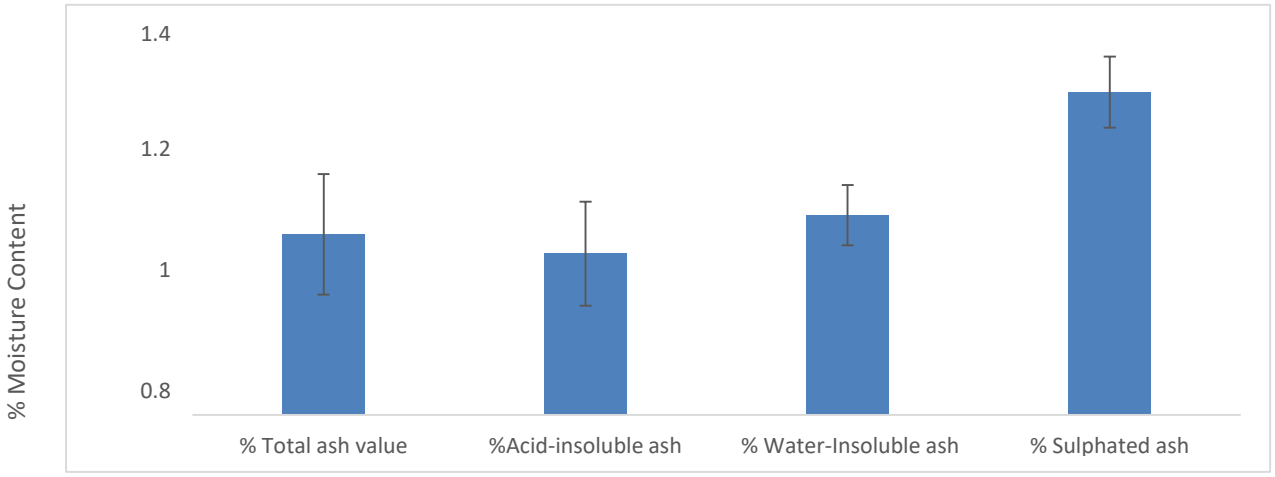


Figure 8.5: Bar graph of the total ash value, Acid-insoluble ash, Water-Insoluble ash, andSulphated ash of leaf of the *Achyranthes aspera.*

Extraction with different solvents using Soxhlet process

In the present study, *Achyranthes aspera* leaves were extracted by using Soxhlet apparatus byusing the five different solvents i.e., Petroleum ether, Chloroform, ethyl acetate, methanol and water (250 mL) as solvent. Results depicted clearly indicate that all the four solvents gave thegood amount of semisolid extract from powdered *Achyranthes aspera* leaves.

In-vitro characterization of extract of *Achyranthes aspera* leaves

Physical observation of Extract of leaf of *Achyranthes aspera*

On visual observation, physical appearance of all prepared extract in different solvents wasshown in table 8.3.

Table 8.3: Physical observation of all prepared extract of *Achyranthes aspera* leaves

Sr. No.	Code	Physical appearance
1	AAP	Light brown colour
2	AAC	Dark yellowish brown colour
3	AAE	Dark yellowish brown colour
4	AAM	Dark yellowish brown colour
5	AAW	Dark yellowish brown colour

Extractive value

The influence of solvent over the formation of extract was determined by the using the extractive value and extraction methods on the extractive values and Karl Pearsons coefficientof correlation values of *Achyranthes aspera* leaves powder were shown in Table 8.4and fig8.6. The extractive values obtained using Soxhlet extraction method in five different solvents i.e., petroleum ether, chloroform, ethyl acetate, methanol and water were found to be in a range of5.72% to 27.31%. The maximum extractive value was found to be with ethyl acetate solvent 27.31% while the petroleum ether revealed the minimum extractive e value 5.72% among all other five different solvents.

Table 8.4: Extractive value of different extract of leaves of *Achyranthes aspera*

S.No.	Code	% Extractive Value
1	AAP	5.72 ± 0.67
2	AAC	16.15 ± 1.45
3	AAE	27.31± 2.95
4	ZAM	16.95± 4.25
5	ZAW	14.87 ± 0.99

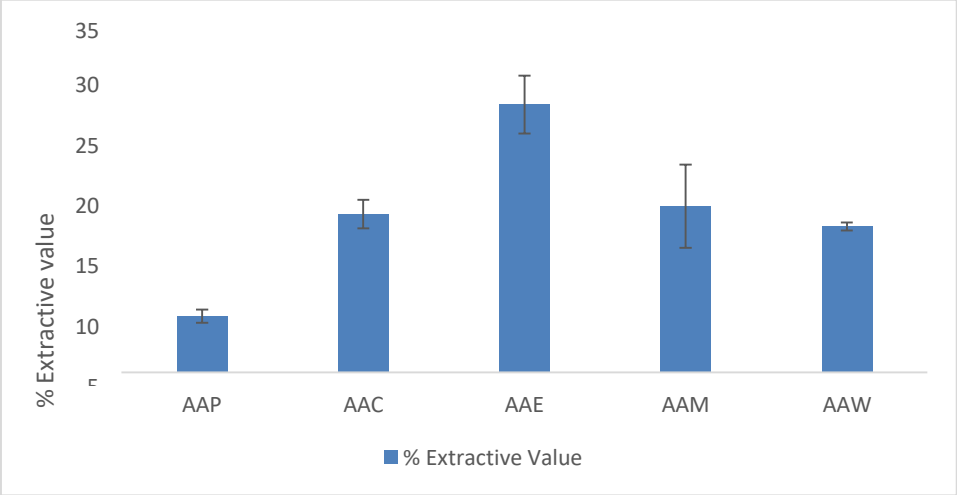


Figure 8.6: % Extractive value of different extract of leaves of *Achyranthes aspera*

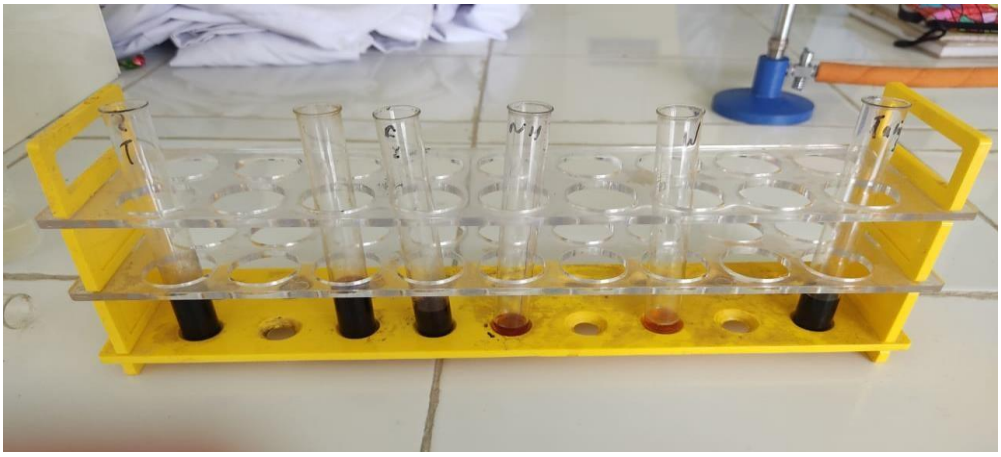
Preliminary phytochemical analysis

Table 8.5: Phytochemical test different leaves extract of *Achyranthes aspera*

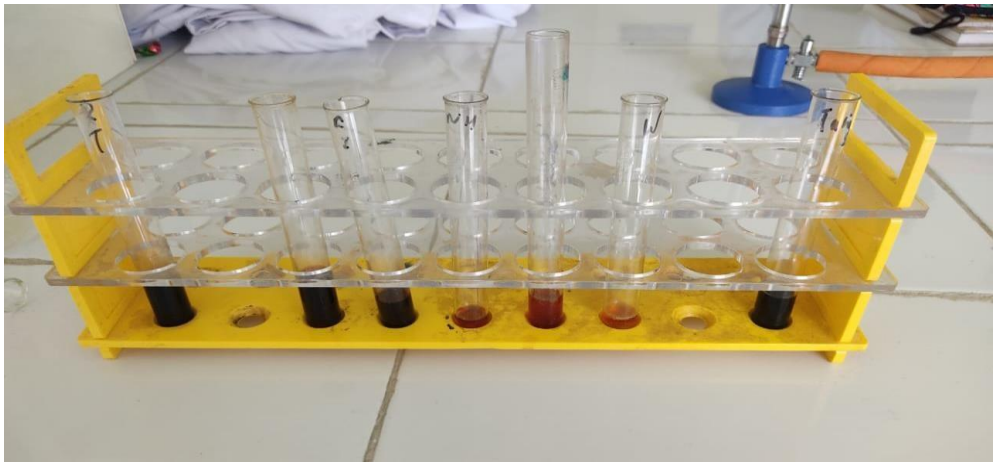
Phytochemical Tests		**Methanolic Extract**	**Aqueous Extract**	**Chloroform Extract**	**Ethyl acetate extract**	**Petroleum ether Extract**
Carbohydrate	Benedict's test	+	+	-	-	-
	Fehling's test	+	+	-	+	-
Proteins and Amino acids	Millon's Test	+	+	+	-	-
	Ninhydrin Test	+	+	+	-	+
Phytosterols	Libermaan -Burchard test	+	+	-	+	-
Glycosides	Brontranger's Test	+	+	-	-	-
Flavonoids	Lead acetate test	+	+	+	+	+
	FeCl3 Test	+	-	-	+	-
Alkaloids	Wagner's	-	-	+	+	+
	Mayer's	+	+	-	+	+
	Draggendrof's test	+	+	+	+	+
Phenolic Compounds	Ferric chloride test	+	+	+	+	+
	Lead Acetate test	+	+	+	+	+
Tannins	Ferric chloride test	+	+	-	+	+
Saponin	Foam test	+	+	+	+	+
Triterpenoids	Salkowasky test	+	+	+	-	+
	Hishron test	+	+	+	-	+
Gum and Mucilage	Alcohol test	-	-	-	-	-

+ =Present; - =Absent

In the present study, the phytochemical screening was performed the sequential extraction of some solvents such as petroleum ether, ethyl acetate, chloroform, methanol and distilled water extracts of *A. aspera.* Phytochemical analysis of different chemical compounds (alkaloids, terpenoids, phytosterol, tannins, saponins, flavonoids, phenols, glycoside, amino acids, gums and mucilage and carbohydrates) were tested in five different extracts.



(a)



(b)

Fig 8.7 Phytochemical test of leaves extract

Antibacterial activity of ethyl acetate extract of the leaves of the *Achyranthes aspera*

The ethyl acetate extract of the leaves of the *Achyranthes aspera* showed good antibacterial activity against almost all four pathogenic bacteria (Fig 14. and table 12). The antibacterial activity was found to be concentration dependent.

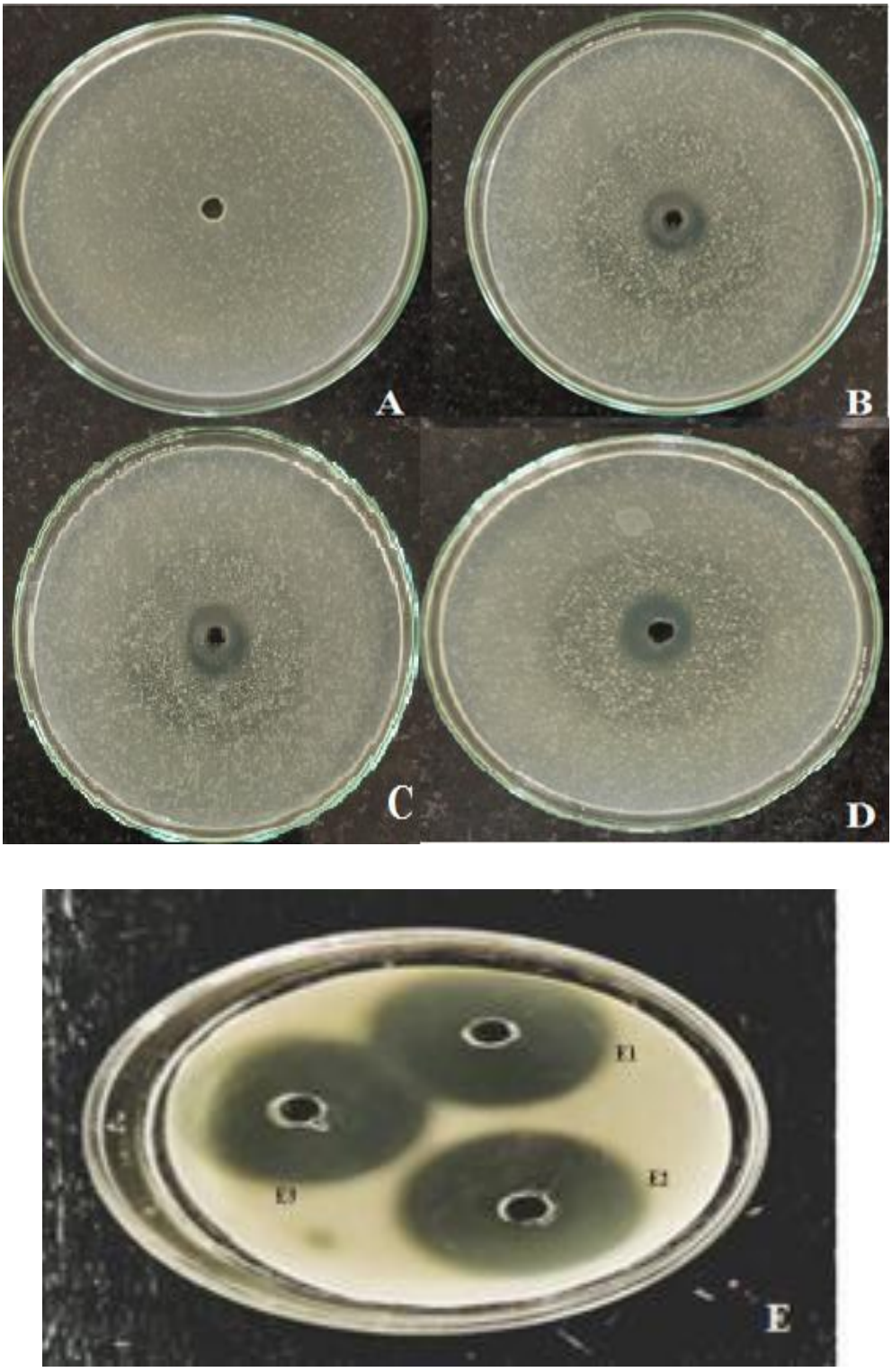


Figure 8.8: Zone of inhibition (mm) of different concentration ethyl acetate extract of the leavesof the *Achyranthes aspera* and positive control against all four bacteria

PC: Positive control, A: 10mg/ml, B: 50mg/ml, C: 100mg/ml, D:200mg/ml, E:300mg/ml

Table 8.6: Antibacterial activity of ethyl acetate extract of the leaves of the *Achyranthes aspera* against all four bacteria.

Extract concentration and Positive control	Bacteria			
	E.coli	P. Aeruginosa	Staphylococcus aureus	Bacillus subtilis
Chloramphenicol (50 µg/ml)	+++	+++	+++	+++
10	-	-	-	-
50	++	+	+	++
100	++	+	+	++
200	++	+	+	++
300	++	+	+	++

+: Activity present, -: No Activity, +++ : Very good activity, ++: Good activity

Table 8.7: Zone of inhibition of the ethyl acetate extract of the leaves of the *Achyranthes aspera* against all four bacteria

Concentration	Zone of Inhibition (mm)			
	E. Coli	P. Aeruginosa	S. Aureus	B. Subtilis
Chloramphenicol (50 µg/ml)	20±1	22.34±1.52	21.67±1.52	21.65±1.49
10(mg/ml)	0±0	0±0	0±0	0±0
50(mg/ml)	3.6±1.15	5.51±2.51	0.72±1.15	8.0±1
100(mg/ml)	7.67±0.58	9±1.02	4±1	12.34±2.08
200(mg/ml)	11.68±1.16	12.64±1.10	6.35±0.57	14.31±0.58
300(mg/ml)	13.35±2.08	13±2	8.35±1.50	16.70±1.55

In 300mg/ml concentration of the ethyl acetate extract of the leaves of the *Achyranthes aspera*, the highest inhibition zone of 17.60±1.55 mm diameter clear zone was noticed against B. Subtilis and 14.35±2.08 mm clear zone recorded against E. Coli. The lowest inhibition zone of 8.36±1.50 mm was observed against S. Aureus. At concentration 10 mg/ml, no activity was observed against all four bacteria.

MIC value

MIC values for ethyl acetate extract of the leaves of the *Achyranthes aspera* against all four bacteria E coli, P. Aeruginosa, S. Aureus, Bacillus Subtills, were recorded as 24±1.0, 19.30±1.50, 33.65±2.07, 11.42±0.58 mg/ml, respectively.

Table 8.8: **MIC value of the ethyl acetate extract of leaves at different concentration against fourdifferent bacteria**

Bacteria	**MIC value (mg/ml)**
E.coli	24±1.0
P. Aeruginosa	18.30±1.50
Staphylococcus aureus	32.66±2.07
Bacillus subtilis	10.40±0.57

9. SUMMARY AND CONCLUSION

The present study deals with the phytochemical investigation, in vitro antibacterial activity of *Achyranthes aspera* leaves. Plant *Achyranthes aspera* is a herbareous plant which is found in altitude of 2.0 to 1000m and mainly collect in the month of August to October. *Achyranthes aspera* grow upto in height. Leaves of the plant elliptic ovate and 22 cm long and 2.5 in broad, Stems are square in shape, around 30 cm long florescence with white orred flowers 7 mm broad. The flowers having growth in summer.

T.S of leaf of *Achyranthes aspera* showed that the leaf is dorsiventral and hypostomatic. The lamina shows a single layered epidermis on the upper side composed of cubical cells. The upper epidermis shows mostly uni, bi and multicellular hairs. Glandular hairs are rare. The epidermisis followed by a layer of hypodermis which is usually 3-5 layers of cells thick and is interruptedat places by the palisade layer. The cells of lower epidermis are cubical in shape mostly with unitricellular trichomes. Three vascular bundles are scattered in ground tissue consisting of thinlayered parenchymatous cells. Vascular bundle consists of xylem vessels, trachids and xylem parenchyma, Phloem consists of sieve tubes, companion cells and phloem parenchyma and pericycle. The pericycle is made of 2-3 layers of thick-walled, nonlignified cells.

Moisture content of Achyranthes aspera plant was found to be 0.49±0.47. Total ash found 0.66±0.22, aid insoluble ash 0.59±0.19, and water insoluble 0.73±0.11, sulphated ash 1.18±0.13. Extraction process of *Achyranthes aspera* was done with the help of Soxhlet apparatus or assembly by using the different solvents that are chloroform 16.15±1.45, Petroleum ether5.72±0.67, ethanol 27.31±2.95, methanol 16.95±4.25, and water 14.87±0.99.

Phytochemical screening was performed with the sequential extraction of some solvents such as chloroform, petroleum ether, methanol and distilled water of plant *Achyranthus aspera*. Phytochemical screening of different chemical compounds such as alkaloid, amino acid, tannin, saponins were tested in five different extracts. Various test to check the presence of thecompounds are being done.

The antibacterial activity are done with the these bacteria E coli, P. Aeruginosa, S. Aureus, Bacillus Subtills are done at different concentration. The zone of inhibition value was checkedat different concentration. In concentration 300ug/ml the zone inhibition value was found E.coli, 13.35±2.08, with P.Aeruginosa was 3±2, with S.Aureus was8.35±1.50, with Bacillus Subtills was 16.70±1.55. Similarly at concentration 200ug/ml E. coli value was 11.68±1.16 for P.Aeruginosa 12.64±1.10 and B. Subtilis value was 14.31 ± 0.58. At the concentration 50ug/ml the zone of inhibition value for the bacteria was 3.6±1.15, 5.51±2.51, 0.72±1.15, 8.0±1. At theconcentration of 10ug/ml no activity was observed against all four bacteria.

10. CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation**.**

11. ACKNOWLEDGEMENT

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