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STUDIES ON ANTIBACTERIAL PROPERTIES OF ACHYRANTHES ASPERA L. AGAINST THE PATHOGENS OF URINARY TRACT INFECTION.

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ABSTRACT

In this present study various extracts of polysaccharide, lipids, polar compound, alkaloids, quaternary alkaloids, crude methanolic extract by infusion and maceration were obtained. Polysaccharides, lipids, chloroform maceration extract, methanol maceration extract and petroleum ether maceration extract were comparatively in more amounts. While, polar compound, alkaloids, quaternary alkaloids, crude methanolic extract and chloroform, methanol, petroleum ether extract of medicinal plant Achyranthes aspera L. was evaluated for their antibacterial activities against multi drug resistance organisms such as *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Proteus mirabilis Polysaccharide inhibited *Staphylococcus aureus*, *Pseudomonas and Klebsiella spp.* The organic extracts of both the leaf and stem parts of the plants at a concentration of 0.5 mg/ml and their activities were measured by estimating zones of inhibition as produced by antibiotic sensitivity method on Mueller-Hinton agar. The results of this research support the use for further analysis in the treatment of infectious diseases such as urinary tract an infection caused by bacteria and has significant scope for antibacterial research.

KEYWORDS: Achyranthes aspera L., Antibacterial, Antimicrobial Activity.

INTRODUCTION

Herbs plants has been handed from generation to generation for thousands of years. Herbal drugs constitute a major part in all traditional system of medicines. Herbal medicine is a triumph of popular therapeutic diversity. Plants have been used for medicine from time

immemorial because they have fitted the immediate personal need. Plants are easily accessible and inexpensive. It has been estimated that in developed countries such as united states, plant based drugs constitute as much as 25% of total drug, while in fast developing countries such as China and India, the contribution in as much as 80%. Thus, the economic important of medicinal plants is much more to countries such as India than to rest of the world. Herbal medicine have a strong traditional or conceptual base and the potential to be useful as drug in term of safety and effectiveness lead for treating different diseases. (Pandey et al., 2013).

Since, beginning plants were only living beings helping in maintaining health of nature. Man and animal depend upon the plant for their basic needs, the clean air, food, fiber and shelter.

Human being so evolved with the plants over the fast few millions year. Plants and human being share some similarities like some compound performed the same functions in the plant and in human body. Previously primitive man during the struggle for existence in the forest must have encountered the miseries of pain and sickness, sustains injuries and to liberate themselves from these suffering, they might have looked forward towards their natural friends, the plants. But due to over population, urbanization man welcome industrial world and he forgot our past. Thus use of medicinal herb was declined. Now a day again use of medicinal plants is increase because of beneficial properties of medicinal plants. India is a perhaps the largest producer of the medicinal herbs and is rightly called "The botanical garden of the world" (Chaudhary R.D.,1996).

In India Rugveda, Ayurveda, Siddha, Unani are the major system of indigenous medicines. In Ayurveda the information about medicinal properties of plant come down traditionally through several generations of experience and through trial and error by primitive mechanism of mention. In India traditional system of medicine, Ayurveda and Unani system are based on the plants. There are about 200,000 to 250,000 spp. of flowering plants growing on earth which are belonging to 10000 genera and 300 families. Out of these 80,000 species are medicinal. In India 45,000 different plants species are present. Medicinal plant provide biologically active molecule for enhanced activity drug preparation. Which decreases the cost of treatment, side effects of other allopathy drugs. Around 70% of Indian medicinal plants are found in tropical areas mostly in various forest types across. Western and Esterne Ghats, the Vindhyas Chotta Nagapur plateau, Aravalis and Himalyas and although less the 30% of medicinal plants are found in the temperature and higher altitudes. In recent time use of

herbal plant material for medicine for variety of infectious diseases is increased because of pronounced irreversible reaction of modern drugs and increased resistance to currently used drugs for infectious diseases. Most of population can't afford the products of Western Pharmaceutical Industry and they use traditional medicines which obtain form plant material. According to World Health Organization (WHO) more than 80% of the world population relies on traditional herb medicine for their primary health care (Arun Vijayan et. al, 2007).

In India there are many traditional plant are used for medicine. Our first traditional plants which is commonly known as Tulas i.e. ***Ocimum sanctum,*** which is mostly used in cough treatment, treatment of heart diseases, vomiting and as mouth freshener.

Achyranthes aspera L.

Achyrantes aspera L. is an erect herb. It is about 1-2 meter in height. It has a woody base. It is commonly found as weed of way sides on road sides. It is grows as waste land herb everywhere. Stems are angular, ribbed simple or branched from the base. It has stem of tinged purple colour. Its branches are absolutely quadrangular striate, pubescent. It has thick leaves of size .These leaves are oval-elliptic or rounded, which are finely and softly on both sides. The leaves are entire and petiolate. The prtiolate is 6-20 mm long. Seeds of this plant are subcylindricale, truncate at the apex and rounded at the base, seeds are brown in Colour (Kumar A. et .al, 2005; Zafar R., 2009).

Achyranthes aspera L. is found on road sides, field boundaries and waste places as a weed throughout India up to an altitude of 2100m and in South Andaman Islands. The plant is also wide spread in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America.

MATERIALS AND METHODS

Collection of plant material

Leaves of **Achyranthes aspera L.** were collected from Dr. D. Y. Patil ACS College Campus, Pimpri, Pune. The green, fresh leaves of ***Achyranthes aspera* L.,** were collected from near the college area during the month of November 2017. These leaves separated from plants and collected in plastic bags. Those preserved in refrigerator at 4˚C till further use.

Extraction of different compounds of plant material

Fresh plant material was homogenized with the mixture of methanol: water (4:1). This gave filtrate which is used in further extraction procedure. While marc obtained was used for extraction of polysaccharide and lipid.

Preparation of crude plant extract

Fresh plant material was homogenized with the mixture of methanol: water (4:1) in the mortar and pestle. Then it was filtered through Whatmann filter paper No.1. This gave filtrate used in further extraction procedure. While marc obtained used for extraction of polysaccharide and lipid.

Extraction of polysaccharide

After filtration marc was grinding and extracted several times with ethyl acetate and filtered thought Whatmann filter paper No.1, this procedure was repeated for several times residue obtained was nothing but polysaccharides. The filtrate obtained used as a lipid extract.

Extraction of lipid

Filtration which was obtained in above procedure evaporated for overnight at 37˚C after evaporation lipids was obtained.

Extraction of polar compound

The filtrate obtained in step 3.2.1 was concentrated by evaporation at 37˚C and acidified with 1N HCL. It was extracted exhaustively with chloroform to give polar compound. Chloroform extract separated and kept for evaporation, which on evaporation gave moderately polar compound. The acid layer (upper) used for extraction of alkaloids and quaternary alkaloids.

Extraction of alkaloids and quaternary alkaloids

Acid layer which was formed in 3.2.4 basified to pH11 with ammonium hydroxide solution. It was extracted with chloroform: methanol (3:1).The mixture kept steady for separation of

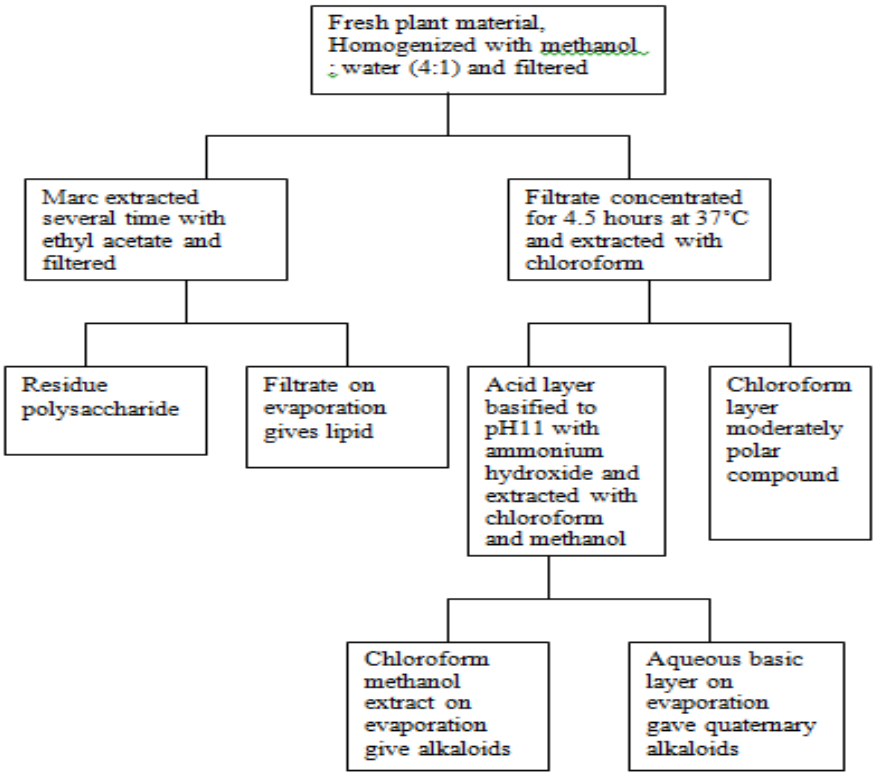
two layers. After separation lower layer on evaporation gave alkaloids, while upper aqueous basic layer on evaporation gave quaternary alkaloids (Kokate C.K. 1997).

Preparation of petroleum ether, chloroform and methanol extract

a. Two extract were obtained by infusion and maceration from 150g dried plant material. The dried leaves was weighted, chopped and extracted with solvent (Londonkar Ramesh et al, 2001).
b. The infusion extract was prepared with 50g of dried leaves in 400 ml of increasing polarity solvent (Petroleum ether, Chloroform and Methanol) respective to their temperature and remove solid matter by filtration method. Filtrate is evaporated for overnight at 37˚C and infusion extract was obtained. Solid matter used for maceration extract.
c. Solid matter obtained in preliminary step was extracted in boiling distilled water at the same condition. The mixture was filtrated through Whatmann filter paper No.1. The filtrate obtained on evaporation for overnight at 37˚C gave maceration extract (Londonkar Ramesh et al, 2001).

Preparation of methanolic extract

a. Methanolic extract was also prepared by using procedure given in Priscilla. (I.U. et al, 2007)
b. 100g of fresh plant material was taken and washed with distilled water. After washing the plant material was chopped in to small pieces and socked in pure methanol for 4 days. After 4 days the plant material was homogenized using mortar and pestle, then the mixture was filtered Whatmann filter paper No.1.
c. The filtrate obtained was further kept for evaporation at 37˚C for overnight and the extract obtained was crude methanolic extract.
d. All different extracts obtained after drying were weighted and recorded. These extract were reconstituted and studied further for antimicrobial activity.



Reconstitution of plant extract

On evaporation of each extract they were weighted and then dissolved in a fixed quantity of solvent. For reconstruction of alkaloid, polar compound, polysaccharide and quaternary alkaloid extract distilled water was used as a solvent, while for lipid and crude methanolic extract, 10% methanol was used as a solvent. For reconstitution of petroleum ether extract, chloroform extract, methanol extract; 5% dimethyl sulfoxide was used as a solvent.

Table No. 01: Protocol for reconstitution of extract of Achyranthes aspera.

Symbol	Name of extract	Amount of solvent added (ml)	Amount of extract added in solvent (gm)	Amount of extract available in solvent (gm/0.1ml)
A	Polysaccharide	1	0.5	0.5
B	Lipid	1	1	0.1
C	Polar compound	1	1	0.1
D	Alkaloid	1	0.5	0.05
F	Quaternary alkaloid	1	0.5	0.05
E	Methanolic extract	1	0.1	0.08
P	Chloroform infusion extract	1	1	0.1
R	Methanol infusion extract	1	1	0.1
T	Petroleum ether infusion extract	1	1.09	0.109

Q	Chloroform maceration extraction	1	0.5	0.05
S	Methanol maceration extraction	1	0.5	0.05
U	Petroleum ether maceration extract	1	0.5	0.05

RESULTS AND DISCUSSION

Collection of plant material

Total of 300g of plant leaves material of *Achyranthes aspera* L. was collected from Dr. D. Y. Patil ACS College, Pimpri, Pune – 411018.

Extraction of plant material

Results of amount of dried extracts obtained from 300 g of plant leaves are as shown in table no.2. Total 12 different types of extracts were prepared from plant material of *Achyranthes aspera* L. The types and quantities of these extracts were as shown in table no.02 and table no.03.

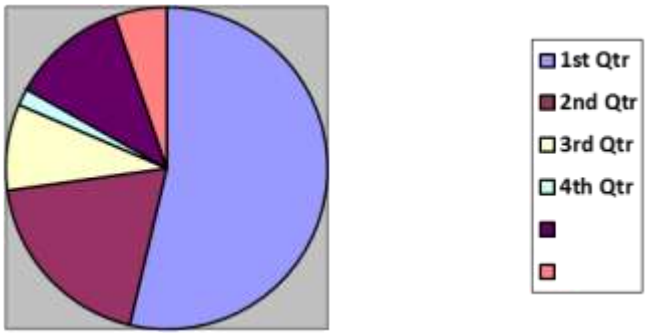


Figure No. 02 – Amount of dried extracts obtained from plant material.

Table No. 02 – Amount of dried extracts obtained from plant material.

Name of Plant Material	**Amount of dried extract (%)**					
Leaves of *Achyranthes aspera*	Polysaccharide	Lipid	Polar Compound	Alkaloid	Quaternary alkaloid	Crude methanolic extract
	18.272	6.50	2.945	0.580	3.942	1.788

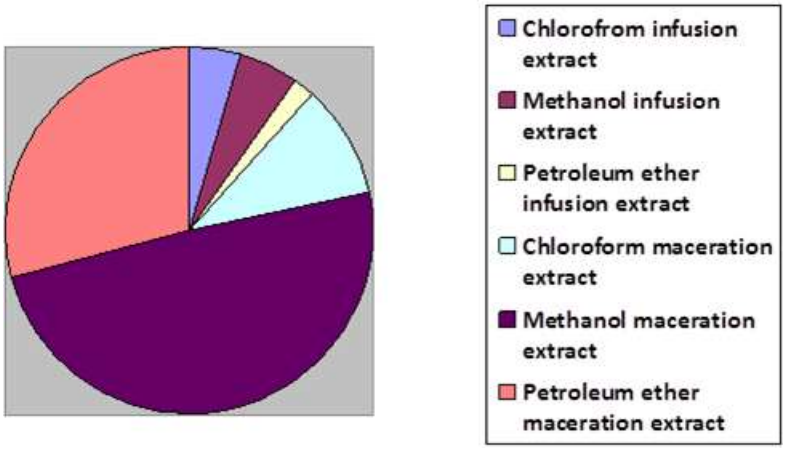


Figure No. 03 – Amount of dried extracts obtained from plant material.

Polysaccharide extract

Polysaccharide obtained from the plant was comparatively high. Extract of *Achyranthes aspera* L. leaves 18.272 g of polysaccharide from 100g of plant material.

Lipid extract

100g of *Achyranthes aspera* L. leaves gave 6.504g of lipid.

Polar compound extract

From the table, it was observed that polar compound obtained was 2.945 g per 100 g of plant leaves.

Alkaloid extract

From 100g of *Achyranthes aspera* L. plant leaves the alkaloid extract was very less in amount and it was o.580g.

Quaternary alkaloid extract

100g of *Achyranthes aspera* L. leaves 3.942g of quaternary alkaloids.

Crude Methanolic extract

Crude methanolic extract obtained from 100g of *Achyranthes aspera* L. plant leaves was 1.788g.

Extracts obtained by infusion

Methanolic infusion extract obtained by infusion of 100g of dried leaves of *Achyranthes aspera* was 6.118g. Which was in high amount as compound to other extracts which obtained by infusion in other solvent Chloroform infusion extract obtained by infusion of 100g of

dried plant leaves of plant in chloroform was 5.134g. . Petroleum ether infusion extract obtained from 100g of dried leaves was 2.18g.

Extract obtained by maceration

Methanolic maceration extract obtained by maceration of 100g of dried leaves of plant material in methanol was 57.25g. This extract was in high amount as compared to other extracts.

Extracts obtained from plant leaves

1. **Polysaccharide.**

2. Lipid.

3. Polar Compound.

4. Alkaloid & Quaternary Alkaloid.

5. Crude methanolic extract chloroform and methanol extract.

6. Preparation of petroleum ether, chloroform and methanol extra.

Antibacterial activity

Result of antibacterial activity of extracts of *Achyranthes aspera* L.

Results of antibacterial activity of extracts of *Achyranthes aspera* L. were as give in Figure No. 03 and Figure No. 04.

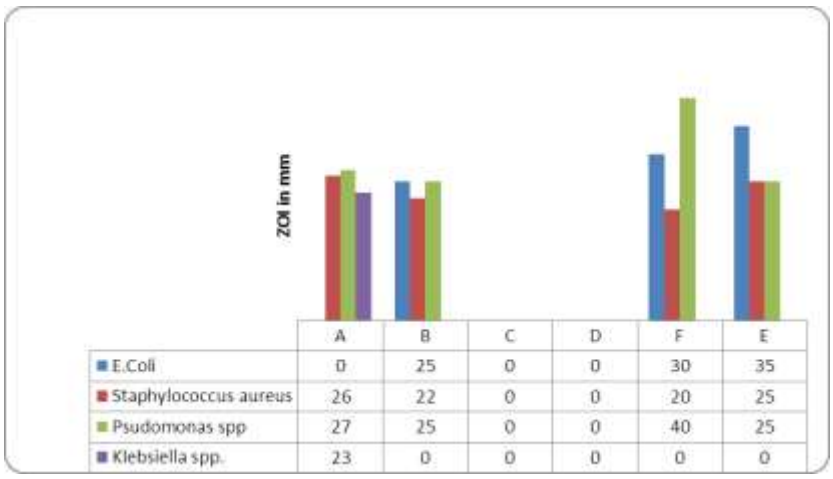


Figure No. 04 – Result of antibacterial activity of extracts of Achyranthes aspera L.

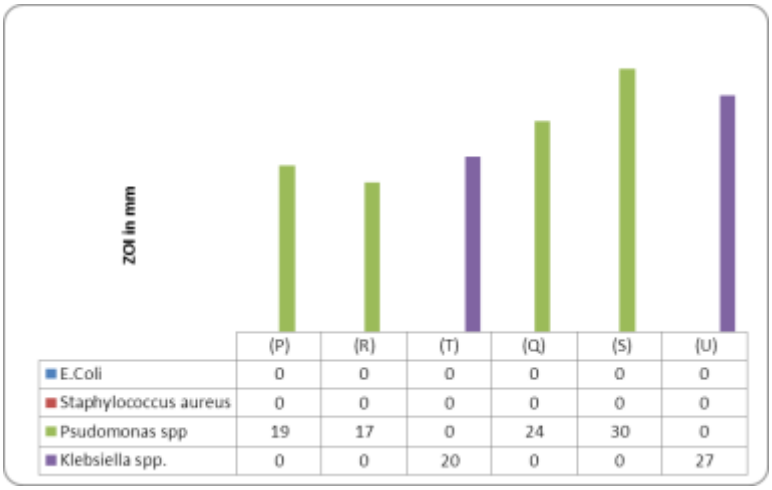


Figure No. 05 – Result of antibacterial activity of extracts of *Achyranthes aspera* L.

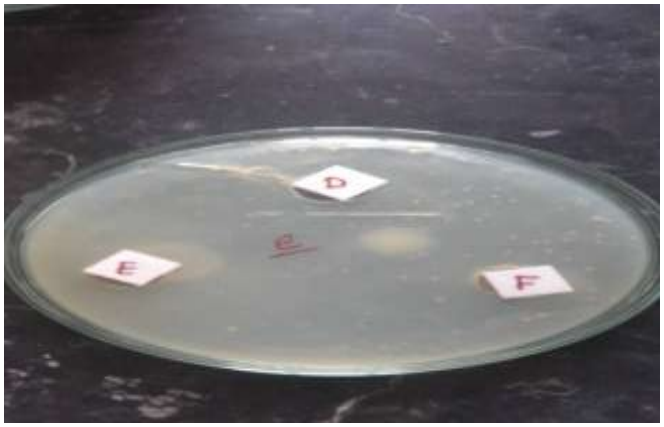
The polysaccharide ,lipid ,quaternary alkaloids, crude methanolic extract, chloroform infusion extract, methanol infusion extract, petroleum ether infusion extract, chloroform maceration extract, methanol maceration extract, petroleum ether maceration extract showed same antibacterial activity. Polysaccharide inhibited growth of Pseudomonas spp, Klebsiella spp. and Staphylococcus aureus. Lipid extract inhibited growth of E.coli, Pseudomonas spp and Staphylococcus aureus. Crude methanolic extract inhibited growth of E coli, Staphylococcus aureus, Pseudomonas spp. Quaternary alkaloid inhibited growth of Pseudomonas spp. E.coli, Staphylococcus aureus .Chloroform infusion extract and methanol infusion extract inhibited growth of Psudomonas spp. and petroleum ether infusion extract nhibition only Klebsiella spp. Chloroform maceration extract, methanol maceration extract inhibited growth of and Psudomonas spp. Petroleum ether maceration extract showed more effect on Psudomonas spp, than on Klebsilla spp .The polar compound extract and alkaloid extract not inhibited growth of E.coli, Staphylococcus aureus, Klebsiella spp. and Pseudomonas spp. Crude methanolic extract and quaternary alkaloid showed maximum inhibitory effect against these pathogenic bacteria. While other extract showed comparatively less effect as compared to crude methanolic extract and quaternary alkaloid extract.

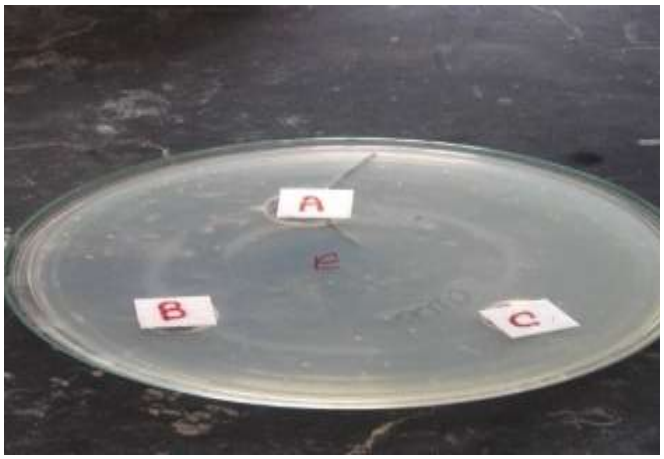
The methanolic, chloroform, petroleum ether extracts of *Achyranthes aspera* L. leaves obtained by infusion and maceration showed inhibitory effect against *Psudomonas spp*. and *Klebsilla spp.*

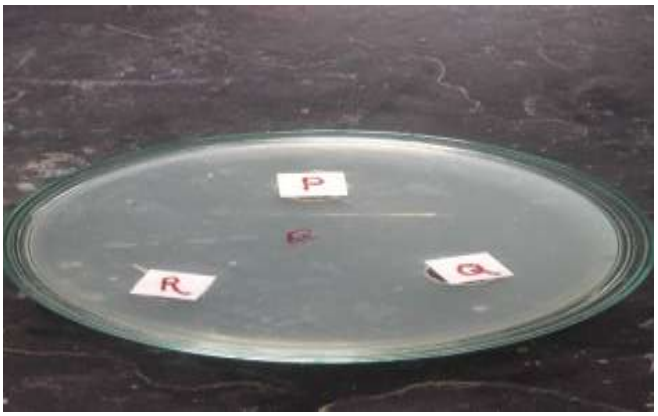
Methanolic infusion extract showed maximum inhibitory against *Psudomonas spp.* Similar result were observed against *Staphylococcus aureus*, by Londonkar Ramesh et al, 2001. They

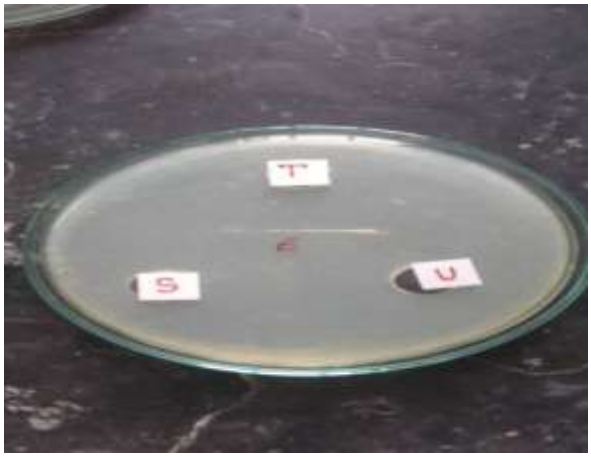
tested methanolic, Chloroform, Petroleum ether extracts of *Achyranthes aspera* L. Leaves obtained by infusion and maceration against 22 microorganisms.

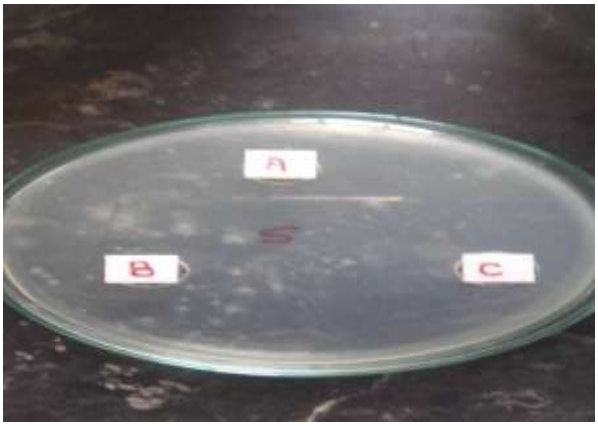
From those extracts only the methanolic infusion extract showed variable degrees of antibacterial and antifungal activities.

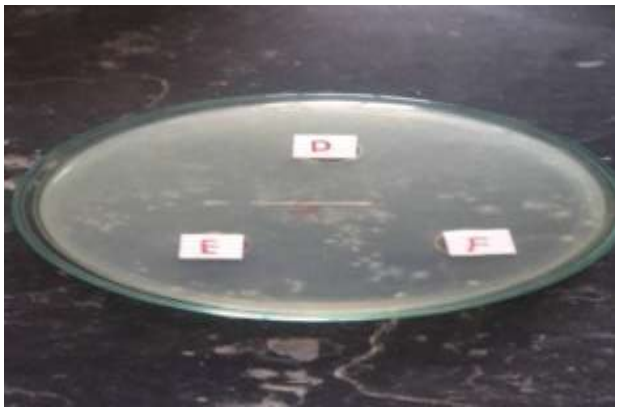




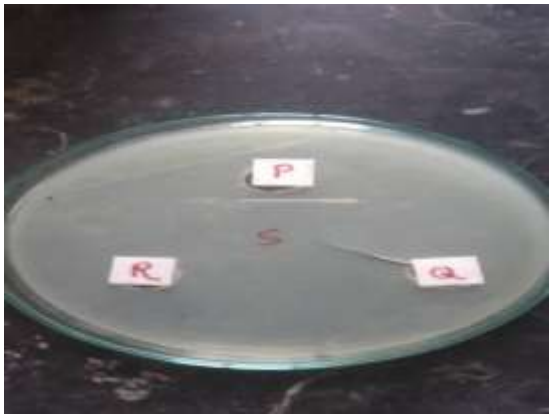








Antibacterial Effect of different extracts of *Achyranthes aspera* L. on *E.coli* and *Staphylococcus aureus* on nutrient agar plates.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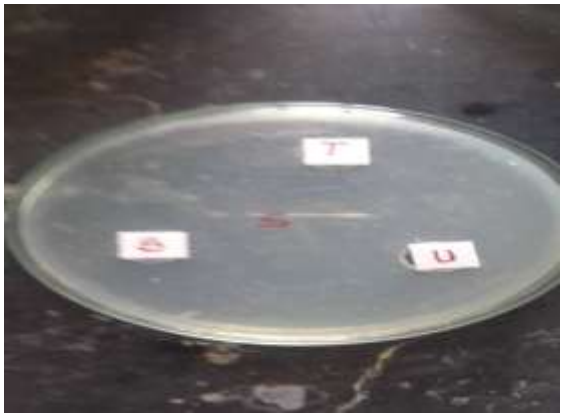
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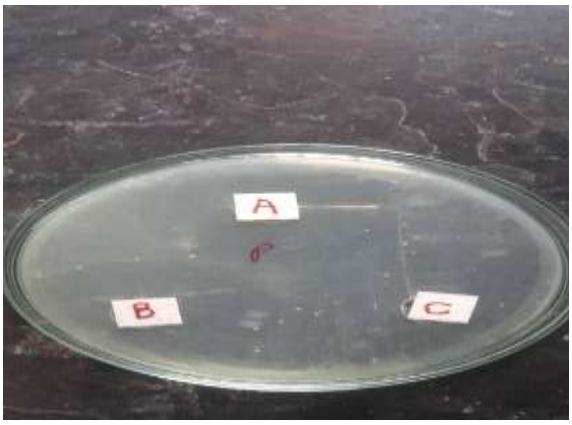
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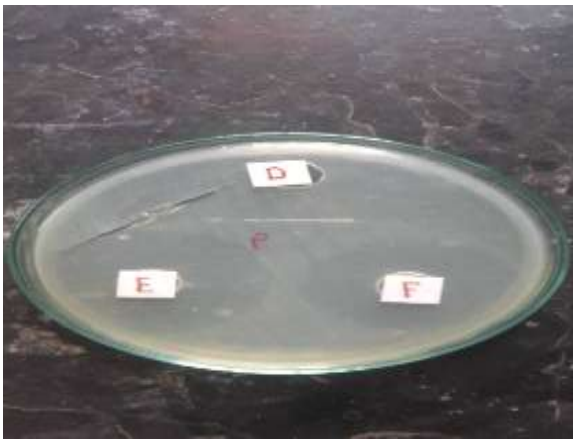
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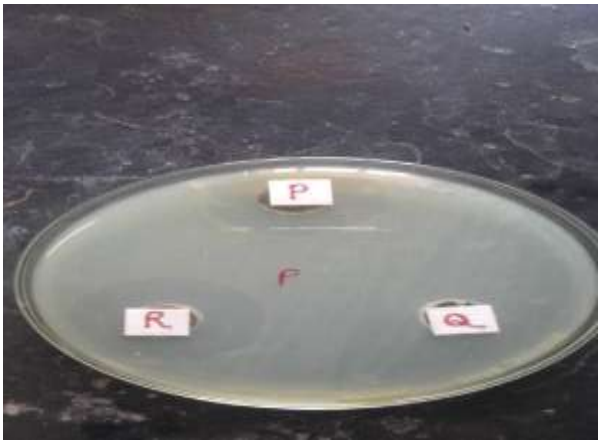
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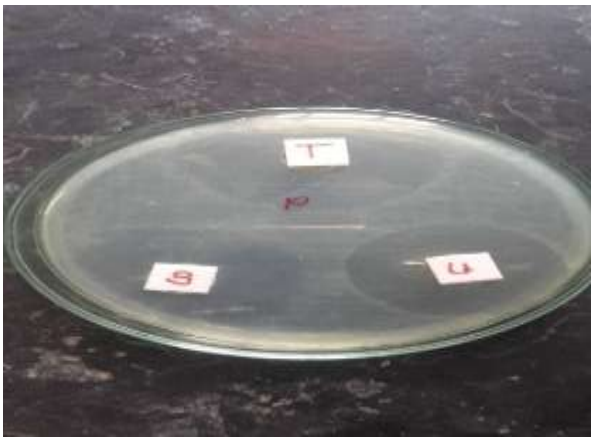
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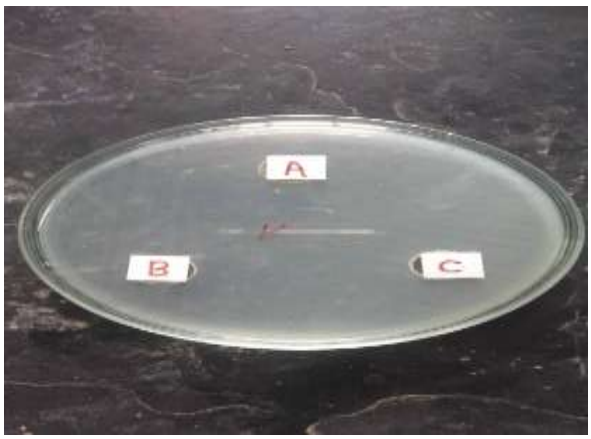
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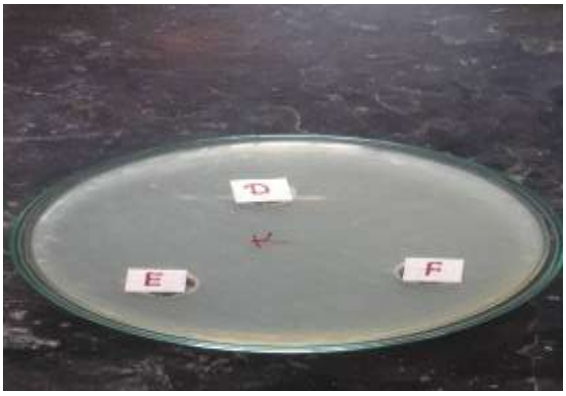
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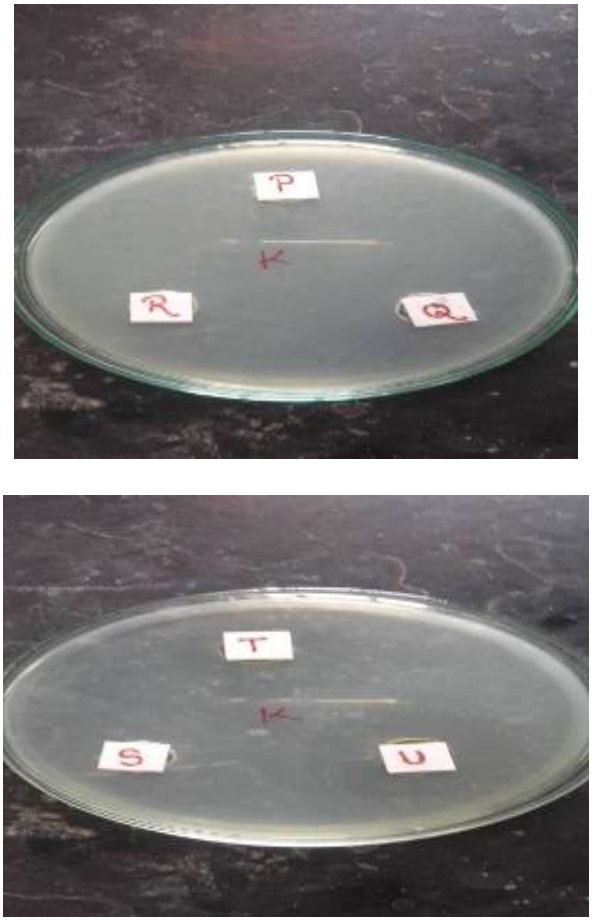

Antibacterial Effect of different extracts on *Pseudomonas spp*. and *Klebsilla spp.*

CONCLUSIONS

Antibacterial properties of its leaves were studied. After collection of plant leaves of *Achyranthes aspera* L., different extract namely polysaccharide, lipids, polar compound, alkaloids, quaternary alkaloids, crude methanolic extract by infusion and maceration were obtained. Polysaccharides, lipids, chloroform maceration extract, methanol maceration extract and petroleum ether maceration extract were comparatively in more amounts. While, polar compound, alkaloids, quaternary alkaloids, crude methanolic extract and chloroform, methanol, petroleum ether extract by infusion were in less quantity.

Lipids, quaternary alkaloids, crude methanolic extract of *Achyranthes aspera* plant leaves were most effective extract that inhibited *E. coli, Staphylococcus aureus, Pseudomonas spp.* Polysaccharide inhibited *Staphylococcus aureus, Pseudomonas and Klebsiella spp.* Chloroform infusion extract and methanol infusion extract were effective against *Pseudomonas* spp. Also chloroform maceration extract and methanol maceration extract were effective against *Pseudomonas* spp. Petroleum ether infusion extract only inhibited *Klebsiella*

spp. And other bacteria were resistant to this extract. Petroleum ether maceration extract inhibited *Pseudomonas* and *Klebsiella* spp.

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