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Heavy metal contents, phytochemical screening and antimicrobial activities of *Astragalus eremophilus*

Hameed Ur Rehman[1,4], Aftab Ahmad[2], Dr Zahidullah Khan[3], Baharullah Khattak[4], Nisar Ahmad[5], Mobeen Kalsoom[2], Haleema Sadia[6], Najeeb Ullah[7], Yasir Ihtisham[7], Summiya Kanwal[8]

[1]*Department of Chemistry, Kohat University of Science and Technology Kohat, KPK, Pakistan*
[2]*Department of Zoology Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan*
[3]*Assistant Professor (Microbiology) Pathology Department Khyber Girls Medical College, Peshawar*
[4]*Department of Microbiology, Kohat University of Science and Technology Kohat, KPK, Pakistan*
[5]*Department of Botany, Kohat University of Science and Technology Kohat, KPK, Pakistan*
[6]*Department of Biotechnology, Balochistan University of Information Technology Engineering and Management Sciences, Quetta*
[7]*Department of Agronomy, Faculty of Agriculture, Gomal University, D.I. Khan*
[8]*Department of Botany, University of Lahore*

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Key words: *Astragalus eremophilus,* Heavy metal, phytochemical screening, Antibacterial activity, Antifungal activity.

Abstract

The aim of the present study was to evaluate heavy metal contents, phytochemical screening and antimicrobial activities of *Astragalus eremophilus* collected from village Inzer Banda District Karak Khyber Pakhtunkhwa Pakistan. During December 2015 to April 2016. For Heavy metal analysis, the plant sample was oven dried, grinded into pieces and digested in perchloric acid and nitric acid. The digested sample was analyzed for selected heavy metals like Fe, Cr, Zn, Cu, Pb and Cd in mg/kg. *Astragalus eremophilus* was screened qualitatively for phytochemicals like flavonoids, terpenes, alkaloids, tannins and saponins. For antibacterial activity agar well diffusion method was used while for antifungal activity tube dilution method was adopted.All the analyzed elements were found below the permissible limits except for Chromium which was noted above the permissible limit (0.06mg/kg). The medicinal plant *astragalus eremophilus* was found rich in carbohydrates, flavonids, alkaloids, tannins, saponins but not in glycosides, when screened for phytochemicals. For antibacterial activity, four strains of bacteria were used, one strain of gram positive bacteria *Staphylococcus aureus* and three strains of Gram negative bacteria *Salmonella typhi*, *Pseudomonas aeruginosa* and *Escherichia coli*. For antifungal activity four strains of fungi *Fusarium oxysporum*, *Alternaria alternata*, *Trichoderma harzianum* and *Rhizoctonia solani* were tested. The result showed that all the fractions of *A. eremophilus* were active against the tested bacterial and fungal strains. The highest activity (21.67±1.117) was shown by an ethyl acetate fraction against all the selected bacteria pathogens. The lowest activity (16.00±0.912) was shown by water fraction against the selected bacterial strains. All the fractions showed significant activity against tested fungi except n-butanol fraction which showed no inhibition to any of the selected fungal pathogens. It was concluded that *A. eremophilus* possesses permissible limit of all heavy metals except chromium, which was above the permissible limit(0.06)mg/kg. The plant showed a high profile of phytochemicals which may be correlated with the potential antimicrobial activity of the plant extract/ fractions. The obtained data were analyzed through statistics Ver.9, by applying one way Anova simple t tests, p valve less than 0.05 was considered significant.

***Corresponding Author:** Hameed Ur Rehman ✉ 03449002451h@gmail.com

Introduction

All living creatures depend upon plants for their food and shelter and treatment. Plants are important in the protection of human life. In medicinal plants there are present many different kinds of chemicals called phytochemicals that produces a specific physiological effect on the humans, plants and animals. Alkaloids, saponins, tannins, glycosides, flavonoids, and phenolic compounds are the important compounds, actively involved phytochemicals [U. Najeeb *et al*,2013]. Those plants that have been used for curing properties are called as medicinal plants. Medicinal plants are classified into different types like shrubs, trees, biennials and annuals, woody perennial plants and climbers.

The extraction and isolation of various types of biological dynamic substances present in medicinal plants started in the 19th century. One of the great achievements in medicinal research is the extraction and isolation of quinine from the bark of Cinchona [A. Muhammad *et al*, 2016]. Medicinal plants are used principally as herbal drugs, fire, mats, shade and also for food [J. D. Phillipson, 1999, A. Dildar *et al,* 2009]. Medicinal plants represent the main source of substances that are active against different microbes, which have antimicrobial activity. This significant use of medicinal plants results in screening of plants for bioactive compounds is, therefore, very much important. Due to the appearance of many drug resistant pathogenic strains and varieties they have got attention [J. J. Rojas *et al*, 2006]. There are immense arguments from traditional healers that modern medicines have more side effects and more expensive than herbal medicines which are less expensive and with least side effects. Low income peoples and small, isolated villages in developing countries use folk medicines for treating many kinds of widespread infections [S. Y. Betül *et al*,2005]. Herbal medication has been enhanced to a good deal in developing countries. It is used as a substitute for health troubles and is getting a good position in pharmaceutical products. Plant is an important source in race of searching new antimicrobial substances due increase in drug resistance in the commonly used antibiotics, used against various human pathogens. Those plants that are used in traditional medication contain an extensive array of compounds. These compounds are used in treating infections as well as in chronic diseases [H. G. Kim *et al*, 2005]. As plants are a rich source of bioactive chemicals, therefore they are used as an alternative to the chemical pesticides, being used against plant pests [M. Daoubi *et al*, 2005, J. D. Phillipson, 2005]. Alkaloids, tannins, terpenes, saponins and flavonoids are essential phytochemicals in plants. According to a survey conducted by WHO, suggests that 80% of the world population depend upon medicinal plants [G. Vines,2004]. The climate of Pakistan favors the growth of medicinal plants, therefore a large number of medicinal plants are found in this region [M.A Khan *et al*, 2012]..*Astragalus eremophilus* commonly known as milk vetch and locally it is called Azmul Kheb is an important medicinal plant, which is a member of the family Fabaceae.*Astragalus* may help protect the body from diseases such as cancer and diabetes. It contains antioxidants, which protect cells against damage. *Astragalus* is used to protect and support the immune system, preventing colds and upper respiratory infections, lowering blood pressure, treating diabetes, and protecting the liver [QY. Yang *et al*, 2011]. Many branches from the top of the root-stock, albo-pilose, hairs spreading. Stipules free lateral, c. 2-3 mm long, albo-pilose, hairs spreading. Leaf imparipinnately compound, rachis (including petiole) c. 2.0-5.5 cm long, albo-pilose, hairs spreading, and petiole c. 6-22 mm long. Leaflets 7-13, opposite to sub opposite, petiole up to 1 mm, lamina c. 5-17 mm long, c. 2-5 mm broad, obovate-broadly elliptic, margins entire, tip retuse to truncate, albo-pilose on both sides, hairs spreading. Inflorescence a 1-5-flowered peduncle raceme; peduncle c. 9-42 mm long. Bracts c. 1.5-2 mm long, albo-pilose, hairs spreading. Pedicel c. 1.0-1.5 mm long. Calyx c. 3-5 mm long, albo-pilose, teeth c. 2-3 mm long. Corolla pale sulphur yellow. Vexillum c. 5.5-6.0 mm long. Wing c. 4.0 mm long. Keel c. 5 mm long. Fruit c. 12-25 mm long, c. 3 mm broad, slightly bent to

semilunar, albopilose, hairs spreading; partly bilocular, 20-seeded. Heavy metals are persistent and stable contaminants present in the environment. Unlike other hazardous organic environmental pollutants, they cannot be chemically or biologically degraded by an average. The effect of heavy metals on human health and on the environment has been well established [R.S. Pawan *et al*, 2006]. In the environment the concentration of heavy metals above a legal or high value can be detrimental to a huge variety of living species. Excessive intake of these heavy metals through ingestion, inhalation or by any means by humans can cause serious complications such as cumulative poisoning, damage to the nervous system, cancer and eventual death [Y. B. Onundi *et al*,2010]. Geographically, the environment is a critical determinant of nutrient content, especially in medicinal plants. In developing countries, medicinal plants act as alternative medicines and help to solve some common and unusual medical problems faced by local communities. Plants receive inorganic nutrients, which also include heavy metals, from the soil to perform their metabolic activities. Heavy metals may be necessary and may not be necessary. Heavy metals are essential in trace amounts and play an important role in plant and animal nutrition, as well as in metabolic reactions and enzymatic processes. Heavy metals such as from, Zn, Mo, Ni, Co, Fe & Co is essential, where Cr and Cd non-core heavy metal fill [M.H. Chouhan *et al*,2002].For centuries, developing countries use medicinal plants to treat various serious diseases. These medicinal plants still play a very important function to supplement the basic fitness needs. The effectiveness of these plants is due to the presence of any chemically and biologically active substances that have certain physiological effects on the human body. In these biologically active compounds, the most important are flavonoids terpenoid, tannins, saponin, alkaloids, glycosides and phenolic compounds. Therefore, it is very necessary to examine these plants for these biologically active compounds through isolation and characterization. This may lead to the discovery of some new active compounds [A. Sunday *et al*, 2012].In order to treat various diseases, individuals and also communities use different plant species. For a plant to be medicinally important must contain some biologically active compounds called phytochemicals. These phytochemicals produce different physiological effects on human health [A. Sunday *et al*, 2012]. Pakistan is located in an area with sufficient plant resources. In Pakistan more than 1,000 species have been reported, having medicinal values and these medically important plant species are used by different peoples to cure different diseases and illnesses [H. Javid *et al*, 2009].Humans began to explore their environment as a result of their basic needs and requirements. The plant material used for human food and medicine has long been [F. Rauf *et al*, 2012]. Medicinal properties and traditional uses of plants have led scientists to extract effective biological materials for various diseases [N. Rajakaruna *et al*, 2007].

In the 20th century occurred the most important advances in the development of antibiotics. These antibiotics have proven important role in the healing of various ailments. But now men are faced with the problem of resistance of pathogens to these antibiotics. The pathogens resistant increased due to genetic variation in these organisms. Another problem is the misuse of these antibiotics and short life span.

The possible solution is the use of herbal medicines and new compounds that are active and effective ingredients. The researchers can try to explore new compounds for the treatment of infectious diseases due to resistance of microorganisms to the prepared drugs [O. Ragasamy *et al*, 2007]. Medicinal plants naturally contain a number of antimicrobial compounds. They are used because they contain powerful antimicrobial agents. Different parts of plants are used to extract raw medicines. Some of these thesaurus is obtained on a smaller scale and some more widely by local communities. A large number of plants are tested against bacteria and fungi and even there are thousands of plants that have not

yet been tested against these microorganisms [B. Mahesh *et al*, 2008].A fungus is responsible for causing various diseases in humans and is one of the leading causes of death worldwide. It causes inflammation, especially in the skin and mucous membranes of humans [V. Duraipandiyan *et al*, 2011]. *Rhizoctonia solani, Fusarium oxysporum* and *Colletotrichum musae* are plant pathogens that have a wide range of distribution and host diversity [M.L.M.C Dissanayake, 2013]. Infection caused by fungi is usually controlled by chemical fungicide, which is usually toxic because these substances interact with the food chain of healthy organisms [P.D Dellavalle *et al*, 2011].

The growing persistence on industrialization and the gathering on the utilize of agrochemicals and the distillation of Fungicides resistant to harvesting in agriculture, and the proposal for the investigation of new biotechnologies and innovative organized methods [Anonymous,2003]. Plants are known to produce some natural substances that are more effective than synthetic compounds. Extracts from plants contain antifungal and anti-inflammatory substances [N.A Yusuf *et al*, 2007].

The plant contains natural oils that are effective against fungal disease [B. Meena *et al*, 2002].The present research work was carried out with the following objectives. To carry out the phytochemical analysis of *Astragalus eremophilus*. To evaluate the antimicrobial activities of *A. eremophilus* against gastrointestinal bacterial pathogens of human origin and some plant pathogenic fungi. To investigate the concentration of heavy metals in *A. eremophilus*.

Materials and methods

Collection and Identification of A. eremophilus

Samples of *A. eremophilus* were collected from December 2015 to April 2016 from village Inzer Banda, District Karak Khyber Pakhtunkhwa, Pakistan. The plant species was identified in the Department of Botany, KUST, Kohat and the voucher specimen was submitted to the department of Pharmacy Kust Kohat for further reference.

Processing of the Plant

Fresh samples of the plant were washed with distilled water to remove the particles. It was then placed at room temperature for shade dry. The shade plant samples were grinded into fine powder with a grinder and stored for further use.

Estimation of heavy metal contents

Determination of heavy metals like Cu, Cr, Zn, Cd, Pb and Fe in the whole plant was done by using Perkin Elmer Model Analyst 400 atomic absorption (AA) spectrophotometer (Shelton, CT, USA) using standard protocol [S.A. Khan *et al*,2008].

Oven drying

Astragallus eremophilus 2g powder was taken in a Pyrex glass beaker and placed in oven at 1100^{o}C for 2-3 hours to remove the moisture. Again, it was cooled and placed in a furnace at 600-900 ^{o}c for 1hr.

Acid digestion

The ash of *A. eremophilus* was cooled at room temperature and 2ml of Nitric acid and Perchloric acid solution was added and then placed the mixture in an oven to dissolve the plant contents.

Filtration

The acid digested mixture was filtered by Whatman filter paper (No.42) in 25ml of graduated cylinder and sterile distilled water was added to further dilute the solution. The solution was kept in dry, clean plastic bottles and placed for atomic absorption spectrophotometer for heavy metals analysis.

Preparation of crude extract and fractions

Dried plant powder of *Astragallus eremophilus* (2kg) was soaked in analytical grade methanol and was regularly stirred after 24 hrs till 15 days. After that it was filtered and passed through the rotary evaporator under reduced pressure at 45 ^{o}C to obtain the crude extract. The crude extract was dried and then partitioned with n- Hexane, chloroform, ethyl acetate,

n-butanol and water obtained soluble fraction. The crude extract and fraction were subjected to phytochemical analysis and antimicrobial activities.

2.5 Isolation of Phytochemicals

The crude extract and their fractions, of *A. eremophilus* were subjected for the isolation of phytochemicals like flavonoids, terpenes, alkaloids, tannins and saponins as per established protocol [P. Tiwari *et al*,2001].

Phytochemical analysis

For identification of different group of phytochemicals Harbone [J.B. Harbone,1973] method was used

Flavonoids

10ml of ethyl acetate was added to each of the crude extracts and their fractions. The solution was heated for a few minutes and then filtered. The filtrate was mixed with1ml of dilute ammonia and analyzed the yellow color of the solution [S.P. Garg *et al*, 1980].

Tannins

20ml of distilled water was added to each of the crude extract and fractions and heated for 5 minutes and then filtered. 1ml of filtrate was diluted in 5ml of distilled water and 2 drops of 10% $FeCl_3$was mix into it to produce bluish color residue [I. A. Ajayi *et al*, 2011].

Alkaloids

For determination of alkaloids diluted HCl solution was added to crude extract and fractions and filtered. The filtrate was mixed with Hager's reagent to produce yellow color residue [G.E. Trease *et al*, 1989].

Saponins

For detection of saponins the crude extract and their fractions were separately heated with 10ml of distilled water. It was then filtered and allowed for cooling. From each of solution 2.5ml was taken and diluted with 10ml of distilled water and stirrer properly till froth was formed [G.E. Trease *et al*, 1989].

Terpenes

5ml of distilled water was taken and about half ml of each of the extract and fractions was mixed with it. It was stirrer and 4 drops of ethyl acetate were added to each of the solution. It produces bright green color confirm the presence of terpenes [V.vaghesiya *et al*, 2004.

Antimicrobial activity A. eremophilus

For antimicrobial activity four strains of bacteria and four strains of fungi were selected. These strains were obtained from Department of Botany, University of Science and Technology Bannu and Department of Pharmacy Kohat University of Science and Technology, (KUST) Kohat. For antibacterial activity agar well diffusion methods were used while for antifungal activity tube dilution method was applied.

Antibacterial activity

For antibacterial activity agar well diffusion method as adopted by Asghari *et al*, [G. Asghari *et al*, 2006] was applied with slight modification. Nutrient Agar was used for culturing of bacteria. Petri dishes; media and other tools were first autoclave at 121 ^{o}C for 15 mins. In each Petri dish 15ml of nutrient agar was put and placed in position to solidify it. After solidification six wells were made in each Petri dish with sterile borer of 6mm diameter. A small colony of each bacterium was taken with sterile cotton swabs and spread onto the surface of each Petri dish. The crude extract and fractions were poured into respective well. The antibiotic Ciprofloxacin was used as positive control and DMSO was used as a negative control. This experiment was repeated three times and the average value was measured to minimize the possible error. All the Petri dishes were placed in an incubator at 37 ^{o}C and the data of the zone of inhibition was taken after 24hrs.

Antifungal activity

For determination of antifungal activity tube dilution method adopted by Atta ur Rehman *et al*, [A. Rehman *et al*,2001] was used with slight modification. The fungal strains were refreshed in nutrient broth

solution. About 9ml of nutrient broth solution was poured in sterile test tubes and 1ml of crude extract and the fractions were added into separate test tubes. A small colony of each fungal strain was added to each of the test tubes. All the test tubes were placed in an incubator at 25 °C for 48 hrs. DMSO was used as negative control and experiment was repeated three times to minimize the errors.

Statistical aanlysis

All the obtained data were analyzed through statistics Ver.9,by applying one way Anova simple t tests,p valve less than 0.05 was considered significant.

Results

The research work was conducted to explore Heavy metals, phytochemical screening and antimicrobial activities of *A. eremophilus*.

Heavy metal investigation in Astragalus eremophilus

Heavy metals outline of *A. eremophilus* was analyzed. The obtained outcome showed to each selected heavy metal was present in the range of their acceptable limits except chromium 0.003 mg/kg, which was present in its exceeding limit suggested by WHO given in table 1 under exploration.

Table 1. Heavy metal contents in *Astragalus eremophilus*.

S.No	Heavy metals	Concentration in *A. eremophilus* mg/kg	Permissible limit mg/kg	P Values
1	Copper (Cu)	1.256	10	0.4205
2	Chromium(Cr)	0.06	0.03	0.2048
3	Zinc (Zn)	5.96	50	0.4245
4	Cadmium (Cd)	0.003	0.03	0.4365
5	Lead (Pb)	BDL	10	0.5000
6	Iron (Fe)	6.098	20	0.3116

BDL= (Below detection limit).

The results given in Table 1 showed the detection of heavy metals like Copper, Chromium, Zinc, Cadmium, Lead and Iron in *Astragaluseremophilus*. (All these selected heavy metals were measured in mg/kg through Atomic absorption spectrophotometer Perkin Elmer 400).

Phytochemical Analysis of Astragalus eremophilus

Results revealed the detection of different phytochemicals in crude extract as well as in various organic fractions of *Astragaluseremophilus* as shown in Table 2.

Table 2. Phytochemical Analysis of *Astragalus eremophilus*.

Extracts	Carbohydrate	Glycoside	Alkaloide	Phenol	Sapnins	Tannin	Flavonide
Crude	+	_	+	+	+	+	+
n-Hexane	+	_	+	+	+	+	+
Chloroform	+	_	+	+	+	+	+
E. acetate	+	_	+	_	+	+	+
N-butanol	+	--	--	--	--	--	-
Water	+	_	_	--	--	--	--

The (+) sign shows the presence and (-) sign shows the absence of.

The data confirmed the presence of Carbohydrate and negative results for Glycoside for all the extracts when tested in favor of alkaloids.

Phenol was found to be present in crude,n-hexane, and chloroform while it was absent in Ethyl acetate,n-butanol and water. For the phytochemicals,like alkaloids, saponin, tannins and flavonids, it was observed that these chemicals were present in all extracts except in n-butanol and water control. (Table

2).

Action of Astragalus eremophilus against the selected strains of bacteria

The results showed the antibacterial action of crude extract and different organic fractions of *Astragalus eremophilus* against the selected bacteria *S.aureus*, *E. coli*,*S.typhi* and *P.aeruginosa*as shown in Table 3.

Table 3. Antibacterial activity of crude extracts and fraction of *Astragalus eremophilus* (mm).

Bacterial strains	Mean Zone of Inhibition (mm)						
	Control	Crude extracts and fraction of *Astragalus eremophilus*					
	Ciprofloxacin, 5µg/Disc	n-Hexane 15 mg/ml	Chloroform 15mg/ml	Ethyl Acetate 15 mg/ml	n-Butanol 15mg/ml	Water 15mg\ml	Methanol 15mg/ml
E .coli	28.67±1.150	17.67±0.933	16.67±0.528	17.00±1.000	14.67±0.771	7.5±0.120	17.33±0.250
S. aureus	30.33±0.310	15.67±1.177	18.00±0.577	20.00±0.255	17.67±1.002	8.3±0.701	20.00±0.150
S. typhi	29.00±1.130	19.00±1.000	20.00±1.158	19.33±1.528	17.67±2.517	7.3±2.932	19.00±1.200
P.aeruginosa	30.00±0.732	19.00±0.606	19.67±0.517	21.67±1.117	17.33±0.082	8.0±0.814	16.00±0.912

P=0.0003 <0.05, Significant.

Antifungal activity of crude extract and fractions of *Astragalus eremophilus* The following results show the antifungal action of methanolic extracts and different organic fractions of *Astragaluseremophilus* against the selected fungal strains *Alternaria alternate,Fusarium oxysporum,Rhizoconiasolani*and *Trichoderma harzianum* as shown in Table 4.

Table 4. Antifungal activity of methanol extract and fractions of *Astragaluseremophilus*.

	Mean Zone of Inhibition(mm)						
Fungi	Clotrimazole	n-Hexane	Chloroform	Ethyl acetate	N-butanol	Water	Methanol
Alternaria alternata	-	+	+	+	--	-	+
Fusarium oxysporum	-	+	+	+	--	+	+
Rhizoctonia solani	-	-	+	-	--	-	+
Trichoderma harzianum	-	+	+	+	--	-	+

Inhibition present (+), Inhibition absent (-).

Discussion

The presence of heavy metals in medicinal plants crosses the WHO limits is a major threat for the people used medicinal plants as drugs. This major threat is more severe in the local village area where people blindly believe on practitioners and do not analyze the medicinal plants for heavy metals [A. Rehman *et al*,2013].

Fig. 1. *Astragalus eremophilus* collected from District Karak, Khyber Pakhtunkhwa, Pakistan.

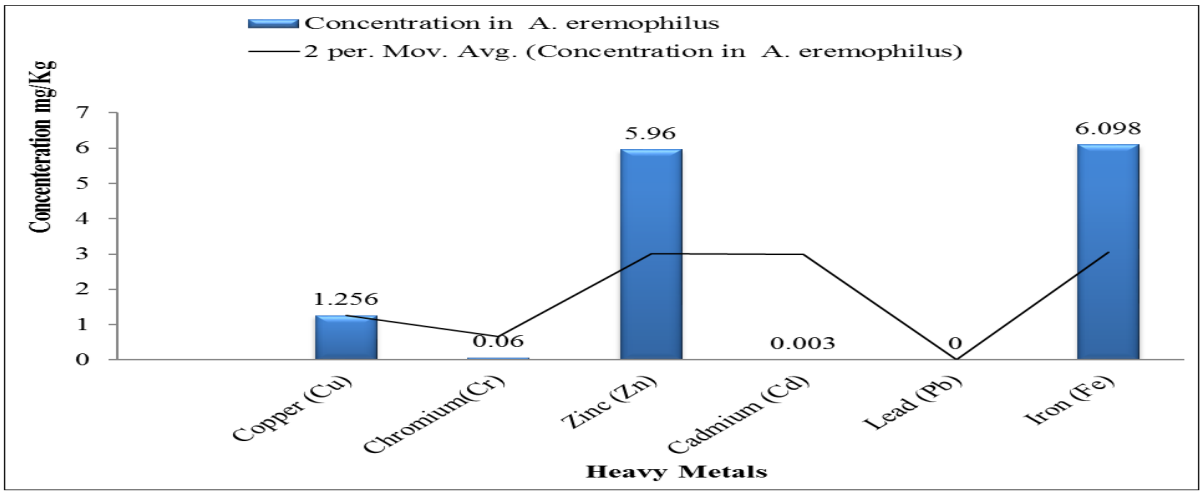


Fig. 2. Heavy metal contents in *Astragalus eremophilus mg*/kg.

The medicinal plant *A. eremophilus* was screened for the selected heavy metals like Fe, Cr, Zn, Cu, Pb and Cd. All the heavy metals were below the permissible limit except chromium (0.06) mg\kg that exceeded the permissible limit (0.03) mg/kg.

i Positive wagner test for Alkaloid shown in Fig.i

ii Positive Shinoda test for Flavonid shown in Fig.ii

ii Positive Ferric chloride phenol test shown in Fig.iii.

iv Positive Foam test for Saponin shown in Fig.iv

v Positive Molish'stest for Carbohydrate shown in Fig.v

vi Positive $FeCl_3$ (iron chloride) test for tannin shown in Fig.vii

Fig. 3. Phytochemical tests in *Astragalus eremophilus*.

The permissible limit of Cr is 0.03 mg/kg in medicinal plants according WHO [A. Niaz *et al*,2013]. From the results it was concluded that *A. eremophillus* enriched in essential metals like Iron (6.098), Zinc (5.96) and copper (1.256).

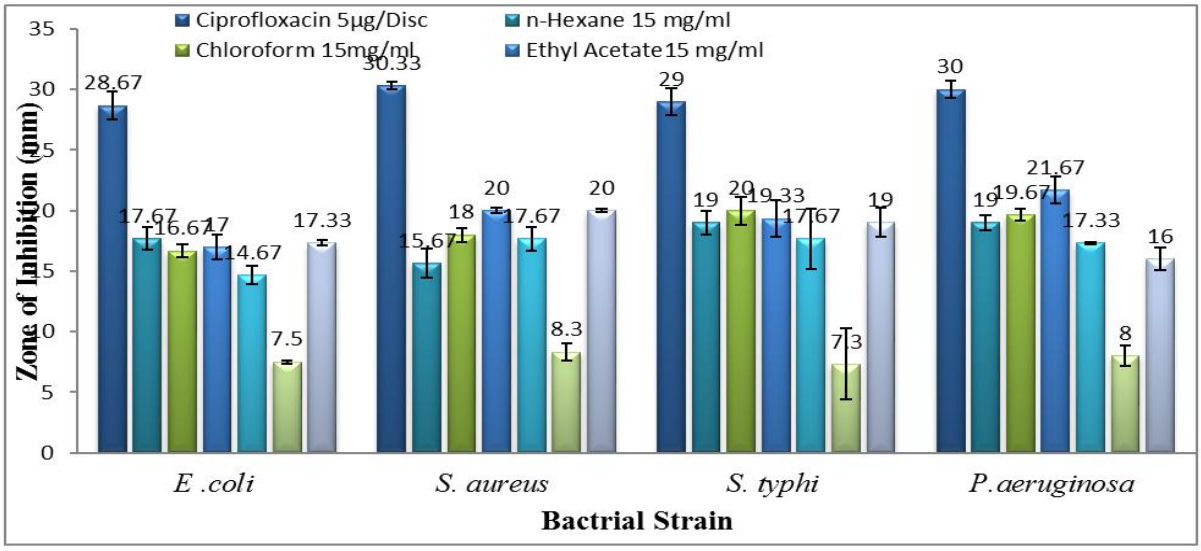
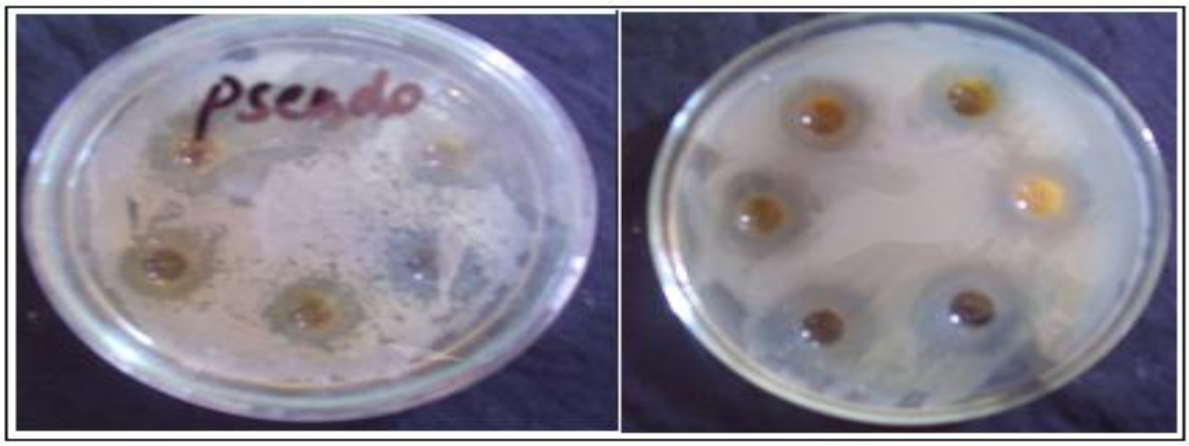
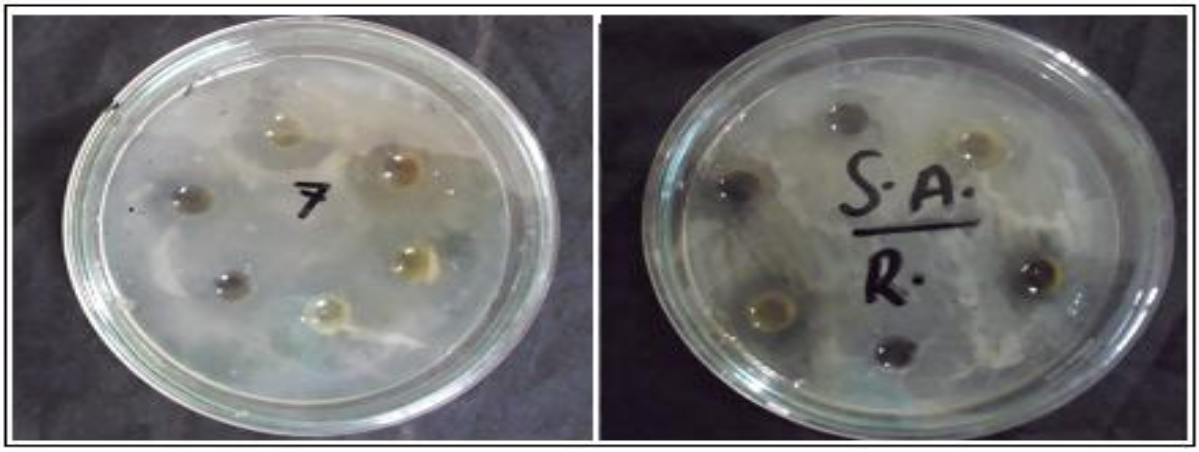


Fig. 4. Antibacterial activity of crude extracts and fraction of *Astragalus eremophilus* (mm).



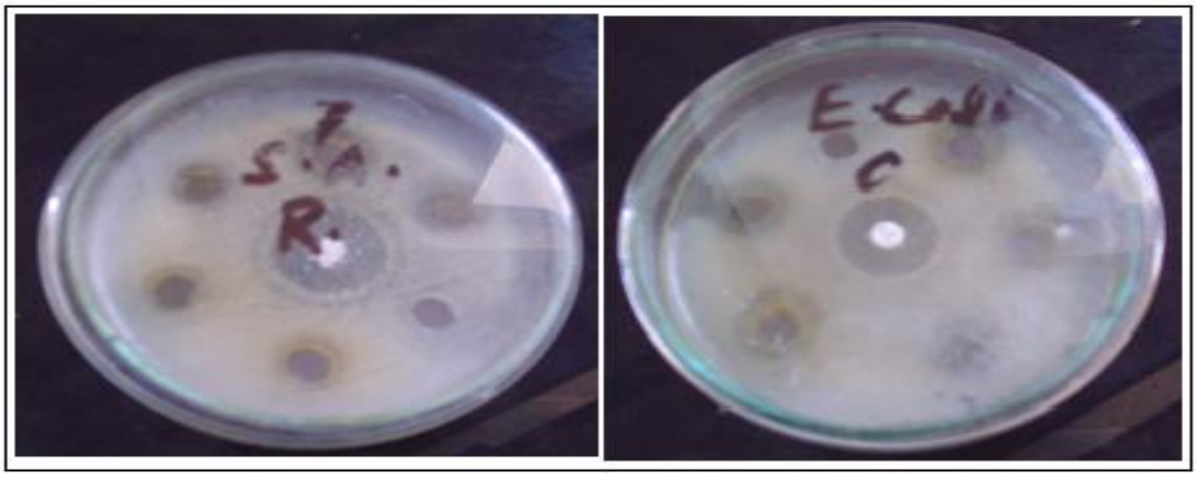
A. eremophilus activity against *P. aeruginosa*. *A. eremophilus* activity against *E. coli*.



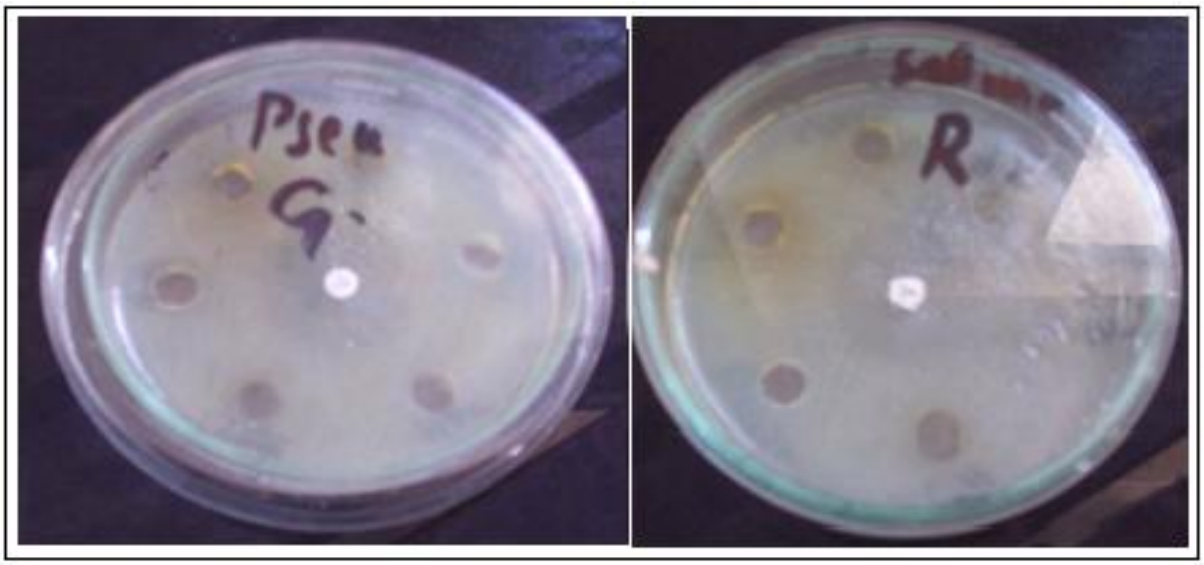
A.eremophilus activity against *S.typhi*. *A.eremophilus* activity against *S.aureus*

Fig. 5. Antibacterial activity of crude extract and fractions of *A.eremophilus*.

The prophecy of antimicrobial activity of Different chemicals mainly depends upon the substances used in taking out procedure. Most plants used as folk drugs require water for dissolving different substances, but results in our exploration are different as it shows that organic substances have greater solubility than water.



Ciprofloxacin activity against *S.aureus*.Ciprofloxacinactivity against *E. coli*.



Ciprofloxacin activity against *P.aeruginosa*.Ciprofloxacinactivity against *S. typhi*.

Fig. 6. Control groups Ciprofloxacin 5µg/Disc.

The reason is that it depends upon the polarity and internal nature of the compounds.*Astragalus eremophilus* and their derived fractions were screened for various classes of phytochemicals. All the fractions showed the presence of Carbohydrate and absence of Glycoside.

The crude, n-hexane, chloroform and Ethyl acetate were found positive for n-butanol and water negative when tested for alkaloids. Phenol was present in crude, n-hexane, chloroform and absent in E. acetate, N-butanol and water. Saponin, tannin and flavonids are present in crude, n-hexane, chloroform, Ethyl acetate and absent in n-butanol and water [R. Ullah *et al*, 2015].

The crude extract and their resulting fraction of *A. eromphilus* with the same concentration for getting triplicate results which show different zone of inhibitions were tested for antibacterial activity. *In*

vitro antibacterial activity of crude extract and additional obtained fractions of *A. eremophilus* were performed and compared with typical antibiotics (Ciprofloxacin). For antibacterial activity, four strains of the bacteria Viz; *S. aureus*, *E. coli* , *S. typhi* and *P. aeruginosa* were tested by Agar well diffusion susceptibility method. For antibacterial actions the *A.eremophilus*, its crude extract showed significant activity against all the four tested bacteria strains.

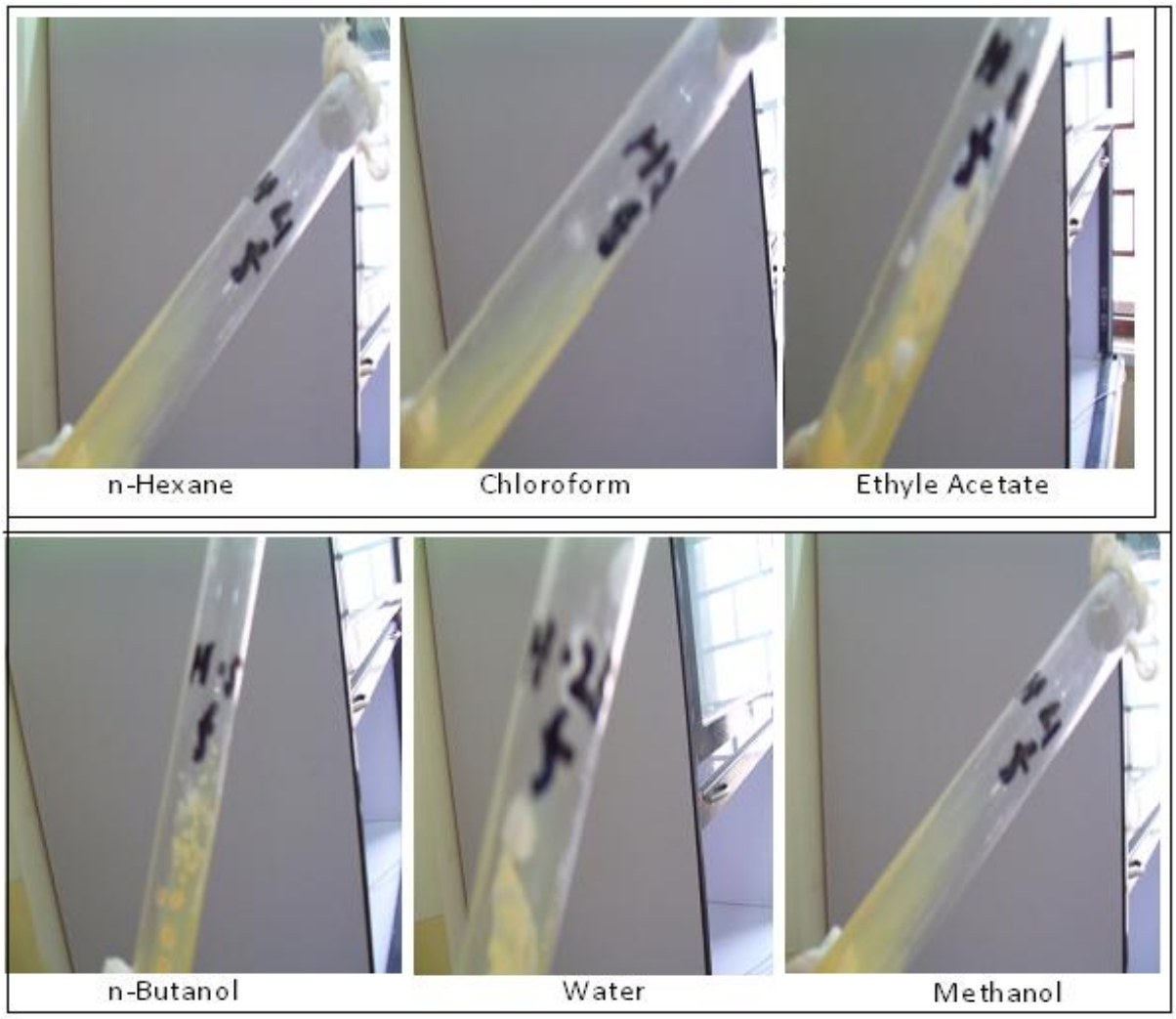


Fig. 7. Effect of Methanolic extracts and different fractions of *Astragalus eremophilus* against *Alternaria alternate.*

The obtained results were comparable with the endeavor of Sen and Batra [A. Sen *et al*,2012].

They exposed the extracts of *Melia azidarach* which showed activity against *E. coli, S. aureus* and *P. aeruginosa*. It shows maximum inhibition against *S.aureus* (20.00±1.15) and *S.typhi* (19.00±3.000) and the minimum inhibition against *P.aeruginosa* (16.00±1.000). The current study was privileged by means of the study of Panghel *et al* [M. Panghal *et al*, 2011].

They proposed that *Pedalium murex* contain highest accomplishment use against *P. aeruginosa*. It has been revealed via the scientist that the natural compounds contain highest antibacterial action than aqua fraction. It is outstanding to the motivation that every one solvent dissolved unlike compounds; *E.coli* (17.33±2.5) followed by n-hexane, chloroform and Ethyl acetate fractions.

The water and n-butanol fractions show minimum inhibition against all the tested bacterial strains. It was distinguished as a result of the effort of Soniya *et al* [M. Soniya *et al*, 2013]. They revealed with the purpose of fuel ethers provide noticeable region neighboring to the disease causing bacteria *E. coli* and *S,. aureus*, at the same time as the aqua and crude fractions did not invent several regions of shyness in opposition to these disease causing agents.

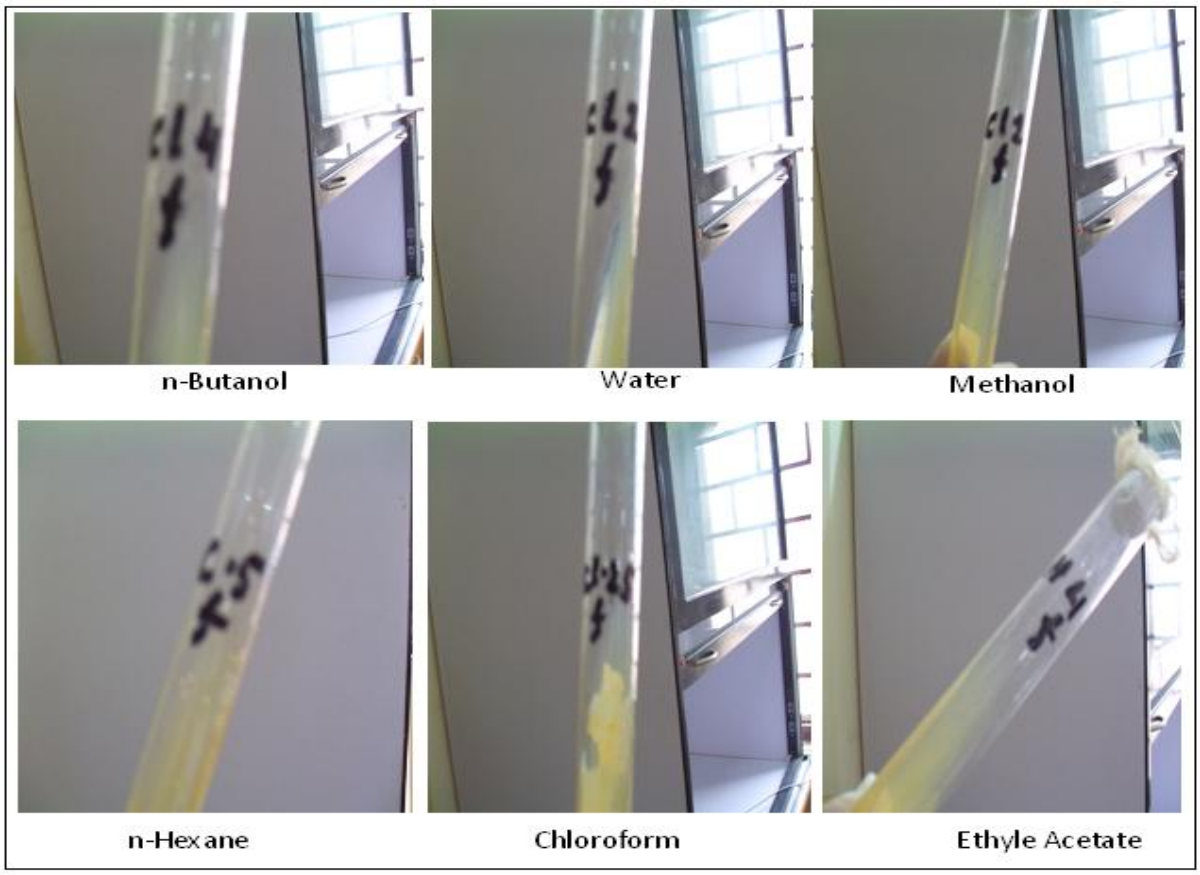


Fig. 8. Effect of Methanolic extracts and different fractions of *Astragalus eremophilus* against *Fusarium oxysporum*.

Antifungal actions of crude extracts plus derived fraction of *A. eremophilus* were performing as well as they were correlated with average Clotrimazol.

In favor of antifungal actions four fungal strains; *Alternaria alternata, Fusarium oxysporum, Rhizoconia solani* and *Trichoderma harzianum* were tested. From the results, it was concluded that the crude and chloroform extracts were more active against all the tested fungal strains than n-hexane. According to Bokhari [M. Bokhari,2009], the crude fraction of lime oil has utmost activity in opposition to plant disease causing fungi like *Alternaria* and *Fusarium*.

The effect showed of *A. alternata* was susceptible to n-Hexane, Chloroform, Ethyl acetate and water fractions. It shows sensitivity to crude fraction. The n-butanol was non active and the water fraction was found only active against the *Fusarium oxysporum*. The similar effect was obtained by the effort of Disanayakke and Jayosinghe [M.L.M.C Dissanayake *et al*, 2013].

They showed that crude extract of natural aromatic plant is extremely useful in the suppression of *F. oxysporum*. It is thought with the purpose of the plant contains definite phenolic compounds which are energetic against different plant fungal diseases.

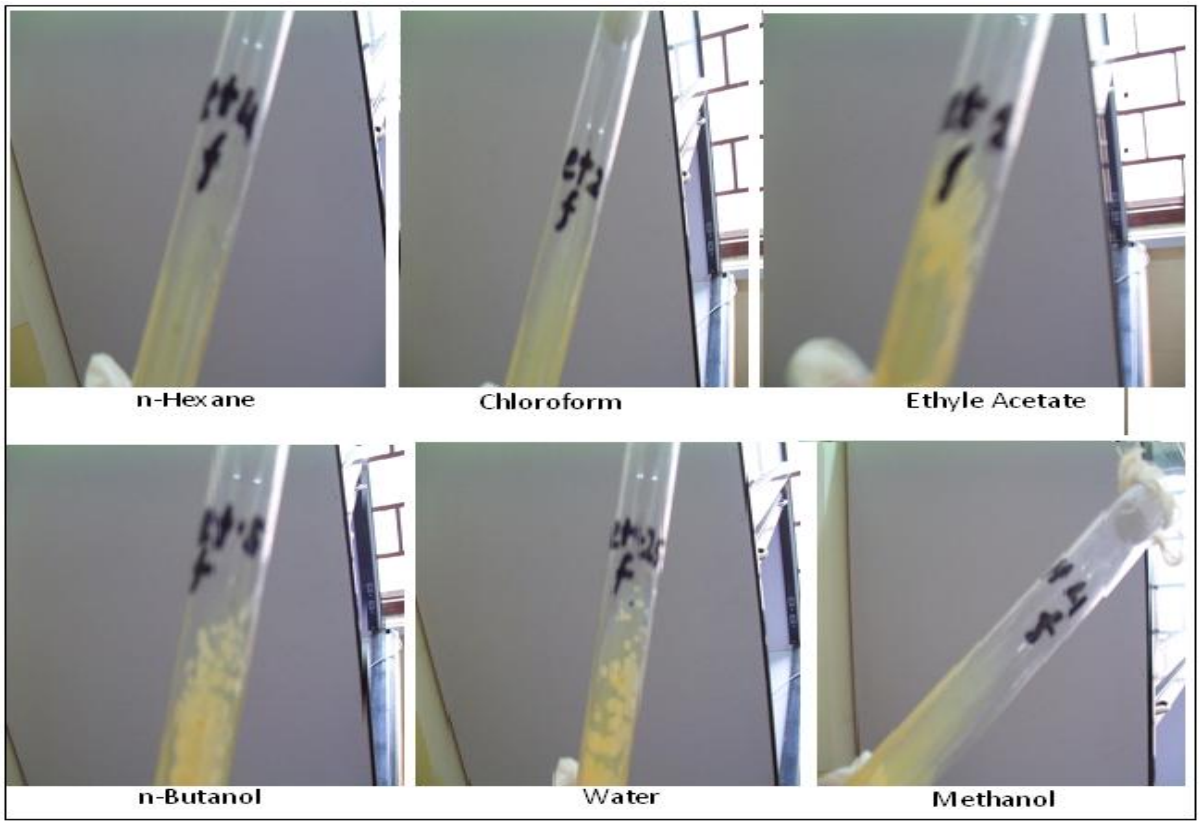


Fig. 9. Effect of Methanolic extracts and different fractions of *Astragalus eremophilus* against *Rhizoctonia solani.*

Fig. 10. Effect of Methanolic extracts and different fractions of *Astragalus eremophilus* against *Trichoderma harzianum.*

The fungus *R. solani* was responsive to Chloroform and water fractions advantage was unwilling to endure fractions. *T.harazanium* was active against n-hexane,chloroform,ethyl acetate and crude while no inhibition was shown by n-butanol and water fractions [A.M.Khan *et al*, 2011].

Conclusions

Traditional herbal extracts of medicinal plants and their recipes were used against microbes and were found therapeutically active.

Astragallus eremophilus was found rich in Alkaloids, Flavonoids and Saponins. Crude extract was more active against the tested bacteria followed by n-hexane and Ethyl acetate fractions.

Antifungal action of crude extract was found the most potent, followed by chloroform and ethyl acetate fractions

The essential elements like Zn, Fe, and Cu were found in high concentration in *A. eremophilus*.

Future recommendations

A.eremophilus possesses strong antimicrobial compounds and therefore the plant is strongly recommended for further studies like isolation of natural products.

The mechanism of action and interaction of these compounds with microorganisms' cell membranes and other cellular parts may be further studied.

Each medicinal plant ought to be analyzed for metals load before processing it for further Pharmaceutical purposes or for local human consumption.

New antibiotics and therapeutics can be prepared from these natural products and may be replaced with medicines showing resistant to microorganisms**.**

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