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Ethnopharmacological and Antibacterial Analysis of Some Selected Pteridophytes Species from Samahni (AJk) Pakistan

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ABSTRACT

In present study, five selected Pteridophytes species were explored for their medicinal potential by using ethnomedicinal, phytochemical and antimicrobial methods and to testify the role as botanic drug and potential source of novel drug discovery. The five plants viz: Adiantum capillus-venerisL., Ptaris vittata L., Equisetum arvenseL., Marsilea minuta L.and Dryopteris serrato-dentata *(*Bedd.*) Hayatawere collected in triplicate from Samahni area of Azad Jammu and Kashmir (AJK) for herbarium specimen and for experimental samples. The ethnobotanical survey analysis congruent with phytochemical profile, which consist of flavonoids, terpenoids, steroid and lipid alkaloid, carbohydrates, proteins and phenols that are effective to cure different diseases. Antimicrobial analysis was conducted in two solvents methanol and chloroform testified by agar cup diffusion method. Four pathogens: Staphylococcus aureus, Bacillus subtilus, Psedudomonas aeruginosa and Escherichia coliwere used for antibacterialactivity screening. The highest activity (19.44±0.58) of methanolic extract was shown against S. aureus while lowest antibacterial potential (10.34±0.02) was explored for chloroform extract. These bryophytic flora can be used for further pharmaceutical analysis and novel drugs can be developed which might potentially more active against resistant bacterial strains. The rich biodiversity of these taxa can be utilized in many indigenous medications and other essential products of life*

Keywords: *Pteridophytes; Antibacterial activity; Phytoconstituents; Ethnopharmacology; Samahni; Azad Kashmir*

INTRODUCTION

Human being has been utilizing plants for their life sustenance in form of food, fodder, medicines and many other necessities of life, since his emergence on the earth. Hitherto tribal and mountain dwellers still depend on plant of the neighbouring environment. Albeit economic and ethnobotanical research have been vastly conducted on angiosperms, but the other group pteridophytes have been ignored at large. The pteridophytes are second largest group, after angiosperms on the planet, which play pivotal role in succession.

It is very clear that pteridophytes make pertinent part of ecosystem and plant communities, as succession is proceeded through these plants. Many plants of pteridophytes group grow on stream banks, slopes of mountains and under shady trees of many areas of the Azad Kashmir (Himalayas region) which produce very interesting results some previous research work. It is explored that forest plants are playing a pivotal role in life of tribal communities.Ferns and their allies are grouped into Pteridophyta with 12,000 species globally present. Pteridophytes being the most primitive vascular plants were dispersed all over the world and many of those now only found in fossil form. These taxa have many vital phytoconstituents which have medicinal potential against many fatal diseases. These have been in used in many traditional herbal medical systems of the earth.

In twenty century, an increase in use of antibiotics and chemical medicines to cure different diseases was seen but these chemical drugs do had exerted side effects on human body and these medicines were also out of approach of huge population of developing countries-being highly cost.

Recently new trend had been emerging about use of botanic drugs, being cheap, easy to get and having no or least side effects on health. The study shows that a large amount of medicinal plants has been used in India due to

their antimicrobial activities and these plants had verified beneficial for humanhood (Manickametal., 2005).In China and Pakistan herbal medicinal systems had been flourishing very fastly with many good outbreaks on human health.

Pteridophytes group namedferns which have ethnomedicinal significance are extensively used by the people of local communities of District Bhimber AJK.Ferns also play an important role in economic significance of country being part of many industrial products. These plants have been used as food, shelter, medicines, fibers, bio fertilizer and insect repellents (Ghosh etal., 2004). In India, medicinal plantsare widely used as folk tonics or in different native medical systems (Brittoetal., 2001).There are many species of ferns in Samahni valley which are used as folk medicines by local peoples. Adiantum capillus-veneris, Pteris vittata, Equisetum arvense, Dryopteris serrato-dentata and Marsilea minuta are reported for their extensive use as folk remedies and proved by studying their antifungal and antibacterial activity. These plants are used for different ailments like tonic and diuretic in treatment of cold, fever, cough and bronchial disorders, as stimulant, emollient, purgative, demulcent, general tonic and hair tonic, in addition to skin diseases, tumors of spleen, liver and other organs.

For few decades, phytochemicals (secondary plant metabolites), with unidentified pharmacological activities, have been comprehensively investigated as a source of medicinal agents (Krishnarajuet al., 2005). Thus, it is expected that phytochemicals with sufficient antibacterial efficacy will be used for the cure of bacterial infections (Parekh etal., 2007). Different phytochemicals have been reported in Adiantum capillus-veneris, Pteris vittata, Equisetum arvense, Dryopteris serrato-dentata and Marsilea minuta like phenylpropanoids, flavonoids, saponins, triterpenoids, carotenoids and carbohydrates (Ansarietal., 2012). The present work was therefore designed: (i) to investigate the phytochemical profile of selected pteridophytes of Samahni area of AJK, (ii) to determine antibacterial activities of its methanol and chloroform extracts against different pathogenic bacteria. These plants can be prove the best efficacy in herbal medicinal use in folklore and traditional medicine system of the indigenous cultures of hilly areas of Samahni Azad Jammu and Kashmir.

MATERIALS AND METHODS

Plant Materials

The plants were collected from different sites of Samahni. Plants were died at room temperature and ground into fine powder by electric blender. The herbarium specimen was also prepared and assigned accession number to it. The herbarium was placed in herbarium of Department of Botany, MUST Bhimber campus.

Organic Solvent Extraction

Powdered plants material of about 25g in dried form was soaked separately in 125ml Chloroform and Methanol. These mixtures were left for 7 days in each solvent at room temperature (25±2◦C). After seven days the extract was filtered in separate beakers (Rawlins and Tindall., 1977). Eventually the extract of each solvent was dried and concentrated by using rotary evaporator then extracts were stored at 4ºC until further test.

Preparation of Dilution

When extracts were dried completely then they were dissolved in their respective solvent in a proportion of 100mg/ml. The concentration of reference antibiotics were erythromycin 5ug and tetracycline 10ug.

Microorganisms

To carry out the antibacterial activity of selected pteridophytes different microbes are used. Staphylococcus aureus are Bacillus subtilus are gram positive bacteria.Gram negative bacteria include Psedudomonas aeruginosa and Escherichia col. These bacteria are used to screened out the antibacterial activity of plant extracts.

Antibacterial Assay

The bacterial stains were collected from biotechnological lab of MUST, Mirpur. A 24 hours old culture of each bacterium was used to evaluate the antibacterial potential of pteridophytes plants. For bacterial growth nutrient agar (28g) medium was used. Agar well diffusion method is used for antibacterial screening; this method is described by (Perez etal., 1966) The sterilized nutrient agar medium. Poring of medium was completed by using plates which were first sterilized at standardized

conditions 121 temperature reached between 40 and 45ºC was poured in the Petri dishes.

Medium Preparation Protocol

For growth of test organism, growth medium was prepared and is used for its development. Prior to the experiment of inoculation of bacterial species, the experimental set up of laminar flow was cleaned using alcohol dipped fore cotton plugs. Then UV light was kept on for 15 minutes for its sterilization of whole chamber. Then the blower and UV lights were turned off and then medium prepared was poured into Petri dishes covering its 66% area. This pouring process was conducted near spirit lamp flame in order to avoid any further contamination.

Then bacterial stains were shifted using inoculating needle which was properly sterilized in order to avoid any contamination from nearby surfaces. The colonies of bacteria were shifted into prepared medium within Petri dishes. Lawn were made on the top of the medium in the whole Petri dishes, then three different wells of the same size in triangular form made within petri dishes. These wells were filled with solvent extract of Pteridophytes for their antibacterial activity against already mention bacteria.For standard, different antibiotics were used that is Erythromycin and Tetracycline. These were placed on the top of medium in the center of Petri dishes by following the disc diffusion method (Karthiketal., 2011). The antibiotics activity was carried out to compare the results of plant extracts of pteridophytes with standard. The plates in which bacterial culture were incubated at 37ºC for 24h. After the completion of incubation period, all the plates were examined and observed the zone of inhibition.

Phytochemical Analysis

Different types of phytochemical tests were performed for the presence of alkaloids, tannins, saponins, flavonoids, steroids, terpenoids and glycosides.This information is presented in the matrix form and analyzed too.

RESULTS

Ethnobotanical survey of Samahni valley was done to collect the information about pteridophytes and their uses through semi structured and structured interview patterns from the local people and information were recorded in the form of questionnaires. Local people of Samahni valley use pteridophytes as folk remedies since long historical background. The indigenous people use these plants for the treatment of various disorderslike to treat cold, fever, cough, bronchial disorders and skin diseases.

Phytochemical Screening:

Phytochemical screening was performed which showed the occurrence of numerous phytochemicals such as: flavonoids, terpenoids, steroid and lipid alkaloid, carbohydrates, proteins andPhenols. These compounds are numerous in methanolic extract as compared to chloroform extract.

Table 1. *Phytochemical constituents in Methanolic extract of the selected Pteridophytes*

Test	*Adiantum Capillusveneris*	*Pteris vittata*	*Equisetum arvense*	*Marsilea minuta*	*Dryopteris serrato dentate*
Alkaloids	+	++	-	+++	-
Flavonoids	_	+	++	+	++
Protein	+++	-	++	-	-
Carbohydrates	-	+	-	++	_
Lipids	+++	++	_	+	_
Phenols	+	-	+++	_	++
Terpenoids	++	++	+++	++	++
Steroid	+++	-	-	++	+++

Key- absent; + present; ++moderately present; +++ strongly present

Table2. *Phytochemical constituents in Chloroform extract of the selected Pteridophytes*

Test	*Adiantum capillusveneris*	*Pteris vittata*	*Equisetum arvense*	*Marsilea minuta*	*Dryopteris serrato dentate*
Alkaloids	+++	-	-	++	+
Flavonoids	++	-	-	+	-
Protein	_	_	+	+	_
Carbohydrates	+	_	_	_	+

Lipids	+	+	++	-	+
Phenols	-	+	-	+	++
Terpenoids	+	-	+	-	-
Steroids	+	++	-	-	+

Key- Absent, + present; ++moderately present; +++ strongly present

Antibacterial Activity

The result of antibacterial activity of five selective fernsAdiantum capillus-veneris, Pteris vittata, Equisetum arvense, Dryopteris serrato-dentata and Marsilea minuta are shown in Tables No.1,2,3,4 and 5.Methanolic extracts of selected pteridophytes show a remarkable activity against four selected microbes while Chloroform extract show minimum against selected microbes, but some species show no activity against bacteria.In antibacterial activity, Methanolic extract of Pteris vittatashow a maximum activity against three bacterial species two were positive and single negative, S. aureus show greatest activity that is 19.44±0.58. E. coli and B. subtilusalso show maximum zone of inhibition that is 12.33±0.33 and 15.21±0.98 respectively. Equisetum arvense also showed maximum activity against S. aureus and B.Subtilus that is 17.99±1.17 and 18.22±0.67 respectively while showed lowest activity 10.34±0.02 against E.coli in chloroform extract.

Table3. *Antibacterial Activity of Adiantum capillusveneris L.*

Zone of inhibition (mm) ± Standard Error means

S.No	Strains	CH	MT	Eryth.	TET
1.	Staphylococcus aureus	10.60±0.029	11.66±0.33	12.33±0.57	27.66±0.57
2.	Bacillus subtilus	11.33±0.19	12.67±0.32	20.00±0.00	38.00±0.00
3.	Psedudomonas aeruginosa	11.31±0.53	13.33±0.38	11.33±0.57	28.00±1.00
4.	Escherichia coli	10.88±0.11	12.42±0.39	18.00±1.00	19.00±0.00

Key- CH= Chloroform MT= Methanol Tet= Tetracycline Eryth= Erythromycin

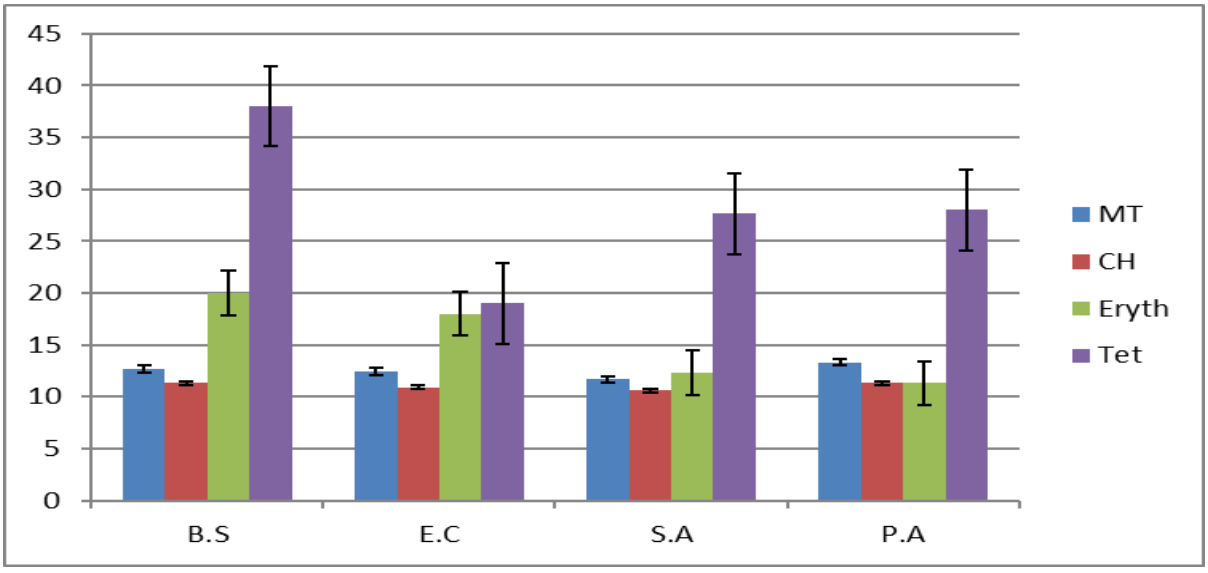

Fig.1 *Graphical representation of Antibacterial activity of Adiantum Cappilus-veneris L.*

Table4. *Antibacterial Activity of Pteris vittata*

L. Zone of inhibition (mm) ± Standard Error means

S.No	Strains	CH	MT	Eryth.	Tet
1.	Staphylococcus aureus	10.6±0.17	19.44±0.58	12.33±0.57	27.66±0.57
2.	Bacillus subtilus	11.08±0.20	15.21±0.98	20.00±0.00	38.00±0.00
3.	Psedudomonas aeruginosa	10.46±0.24	11.88±0.29	11.33±0.57	28.00±1.00
4.	Escherichia coli	11.44±0.40	12.33a1±0.33	18.00±1.00	19.00±0.00

Key - CH= Chloroform MT= Methanol Tet= Tetracycline Eryth= Erythromycin

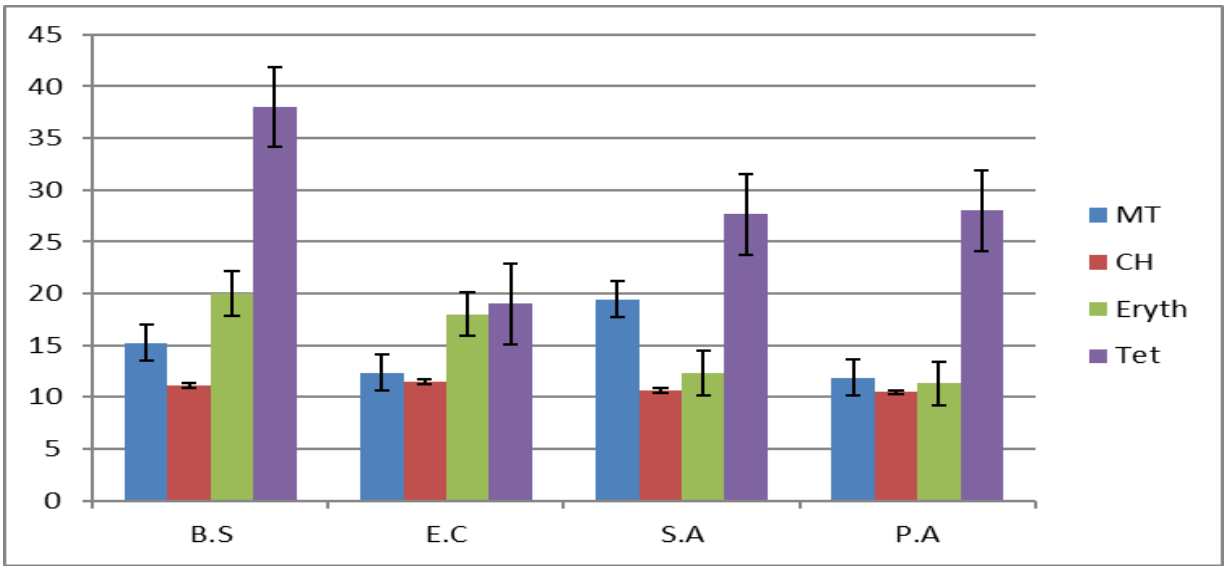

Fig. 2 *Graphical representation of Antibacterial activity of Pteris vittataL.*

Table5. *Antibacterial Activity of Equisetum arvense L.*

Zone of inhibition (mm) ± Standard Error mean s

S.NO	Strains	CH	MT	Eryth	Tet
1	Staphylococcus aureus	14±0.57	17.99±1.17	18±0.58	21±0.00
2	Bacillus subtilus	12.66±0.19	18.22±0.67	19±0.43	19±0.42
3	Psedudomonas aeruginosa	10.83±0.051	11.22±0.77	15±0.63	14±1.00
4	Escherichia coli	10.34±0.02	10.55±0.39	20±0.00	16±0.33

Key- CH= Chloroform MT= Methanol Tet= Tetracycline Eryth= Erythromycin

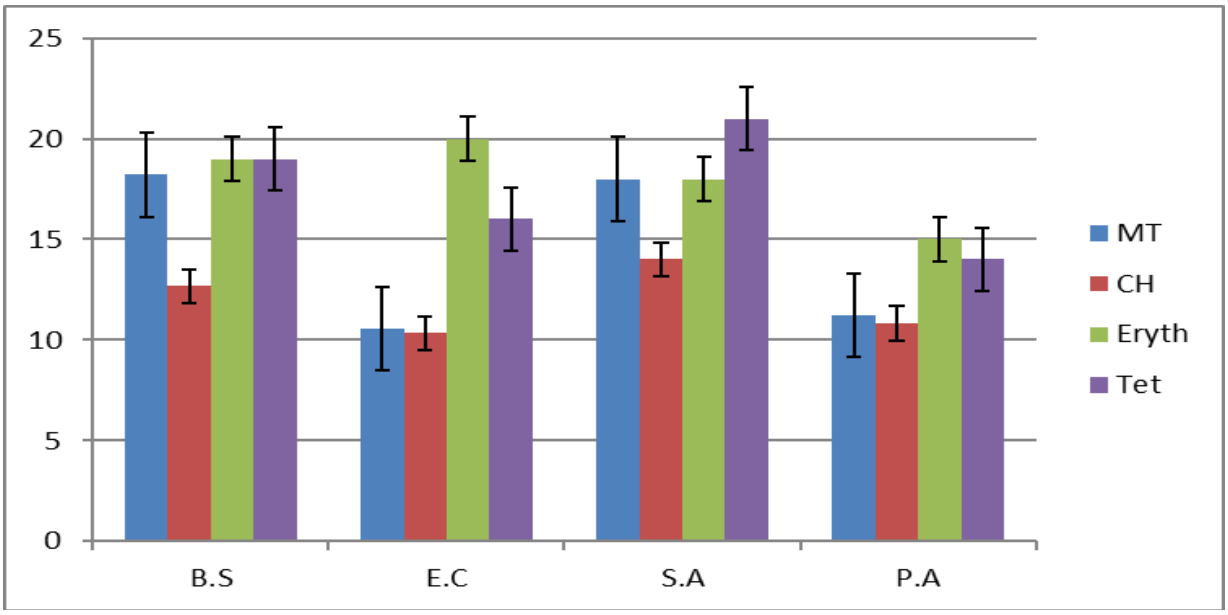

Figure 3. *Graphical representation of Antibacterial activity of Equisetum arvenseL*

Table 6. *Antibacterial Activity of Dryopteris serrato-detata(Bedd). Hayata*Zone of inhibition (mm) ± Standard Error means

S.NO	Strains	MT	CH	Eryth	Tet
1	*Staphylococcus aureus*	14.33±0.06	14.33±0.66	18±0.58	21±0.00
2	*Bacillus subtilus*	12.77±0.72	15.55±0.72	18±0.43	19±0.42
3	*Psedudomonas*	10.48±0.039	12.5±0.28	15±0.63	14±1.00

	aeruginosa				
4	*Escherichia coli*	10.6±0.06	12.33±0.91	20±0.00	16±0.33

Key- CH= Chloroform MT= Methanol Tet= Tetracycline Eryth= Erythromycin

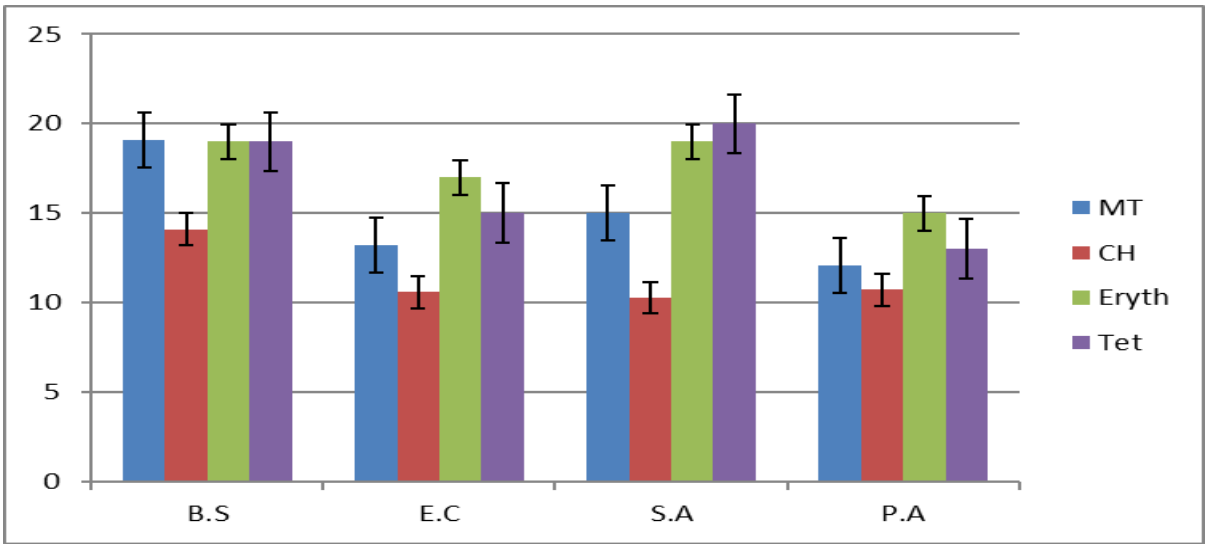

Figure 4. *Graphical representation of Antibacterial activity of Dryopteris serratodentata(Bedd). Hayata*

Table7. *Antibacterial Activity of Marsilea minuta L.*

Zone of inhibition (mm) ± Standard Error means

S.No	Strains	CH	MT	Eryth.	TET
1.	*Staphylococcus aureus*	10.28±0.28	14.99±0.38	19±0.57	20±0.57
2.	*Bacillus subtilus*	14.10±0.39	19.1±1.05	19±0.00	19±0.00
3.	*Psedudomonas aeruginosa*	10.76±0.02	12.11±0.67	15±0.57	13±1.00
4.	*Escherichia coli*	10.6±0.00	13.22±0.67	17±1.00	15±0.00

Key - CH= Chloroform MT= Methanol Tet= Tetracycline Eryth= Erythromycin

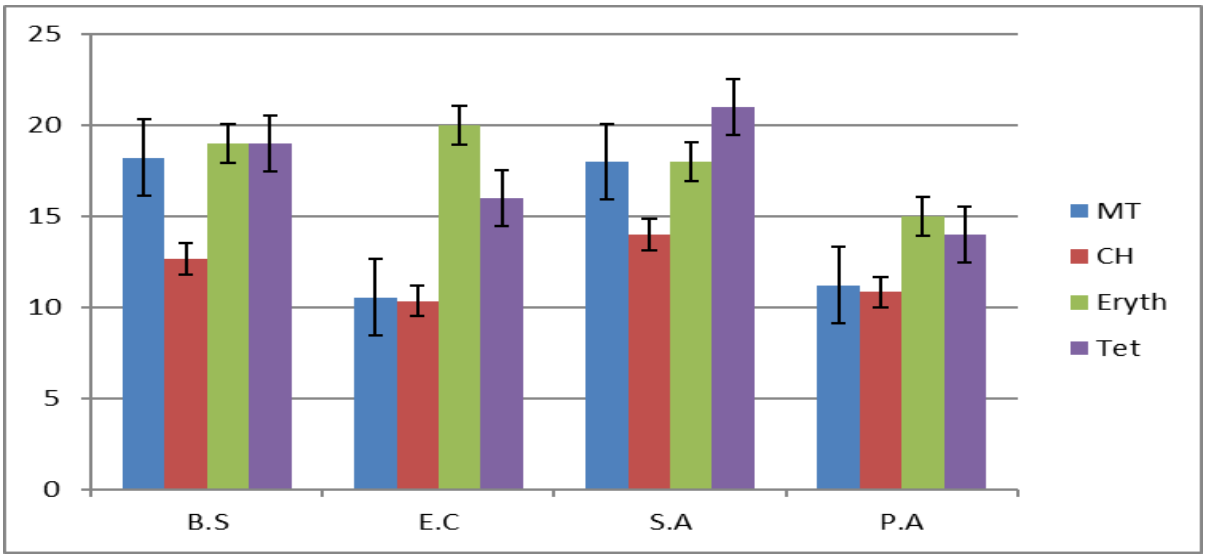

Figure 5. *Graphical representation of Antibacterial activity of Marsilea minuta L.*

DISCUSSION

In the present study five species of pteridophytes were used for testing the antimicrobial activity. Methanolic and chloroform extract were used for this purpose. The plant species were tested against four bacterial strains that are Staphylococcus aureus, Bacillus subtilus, Psedudomonas aeruginosa and Escherichia coli.

Pteridophytes show greater activity against bacterial stains as compare to fungal species. In antibacterial activity, Methanolic extract of

Pteris vittata show a maximum activity against three bacterial species two were positive and single negative, S. aureus show greatest activity that is 19.44±0.58. E. coli and B. subtillus also show maximum zone of inhibition all other extract of pteridophytes that is 12.33±0.33 and 15.21±0.98 respectively. The present study revealed that all species show maximum activity in methanolic extract but in Chloroform extract some species show minute activity .It is common that the sensitivity of Gram negative bacteria is generally higher than that of Gram positive ones. The methanol extract of Adiantum capillusveneris, Pteris vittata, Equisetum arvense, had in vitro greater potential against Aspergilus niger that is 15.3±0.77, 16.25±0.33 and 18±0.33 that is maximum zone of inhibition being against methanol extract. While in Chloroform extract they show minimum zone of inhibition, Equisetum arvense and Pteris vittata show maximum activity against Aspergillus oryzae that is 12.02±0.11 and 12.1±0.35 respectively while other show very rare activity. Pteridophytes show greater activity against bacterial stains as compare to fungal species. In the present study, plants methanolic and chloroform extract were phyto chemically analyzed. Different types of compounds like alkoids, flavonoids, protein, carbohydrate, lipid, phenol, terpenoid and steroids are present. The plants contain high phenol and flavonoid which indicates that the extract have antioxidant effects (Mills and Bone, 2000). These plants' phyto constituents can be used for curing diseases at indigenous level among the local communities and furthermore these can be utilized as source of drug discovery and drug formation for novel products of pharmaceuticals.

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