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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF LEAVES EXTRACT OF *SOYMIDA FEBRIFUGA* A.JUSS.

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ABSTRACT

Rohan, *soymida febrifuga* plant belonging to family meliaceae.The bark of this plant has been proven to have many pharmacological activities. In this experiment the antimicrobial activities of the plant leaves were studied on different bacteria and fungus using different techniques like well diffusion method and minimum inhibitory concentration. In the well diffusion method petroleum ether,chloroform,methanol, water and total aqeous were prepared and tested against some gram positive strains(*B.subtillis,S.aureus*) and gram negative strains(*E.coli*, *P.vulgaris*, *P.aeurginosa*) and were compared with standard(tetracycline).The water(zone of inhibition 23.33) and total aqeous (17.6) gave very significant result to compare with the standard (24) for *S.aureus*. By minimum inhibitory concentration the result on different strains were studied, like *A.niger,A.flavones,P.aeurginosa and C. albicans*. The methanol and water extract gave thebmost significant result the MIC(mg/ml) for water extract against *P.aeurginosa* and *C.albicans* were found to be 1.25 mg/ml.

KEYWORDS: *soymida febrifuga*, antibacterial, antifungal, Minimum inhibitory concentration.

INTRODUCTION

Soymida febrifuga is a tall tree belonging to family meliaceae; commonly known as Indian redwood,bastrol cedar.pharmacologically the plant is of great importance in the ethano-medicinal, use. It contains some important constituents like in bark lupeol,sitosterol,methyl angolensate, leaves contains Quercetin, rutin and fruits abundantly contains tetraterpenoids. The ethano botanical use in treatment of diarrhea,dysentery and fever, as a bitter tonic in general debility,treatment of rheumatic swelling, in gargles, vaginal infection etc.

Antimicrobial studies

Studies involving traditional medicines frequently contribute new ideas. Various species of plants possess antimicrobial activity. Plant derived drugs serves as a prototype to develop more effective and less toxic medicines.

Different methods of antimicrobial screening

1. Diffusion method: In this technique, a reservoir containing plant extract to be tested is brought into contact with an inoculated medium (e.g. agar) and, after incubation; the diameter of zone around the reservoir (inhibition diameter) is measured. Different types of reservoirs have been employed, including filter paper discs, porcelain or stainless steel cylinders placed on the surface and holes punched in the medium (well- diffusion).

2. Dilution method: In this method samples being tested are mixed with a suitable medium that has previously been inoculated with the test organism. After incubation growth of the microorganism may be determined by direct visual or turbidimetric comparison of the test culture with a control culture. Usually a series of dilutions of original sample in the culture medium is made and then inoculated with test organism. After incubation, the end point of the test is taken as the highest dilution, which will just prevent perceptible growth of the test organism (MIC VALUES).

3. Bioautographic method: This method is used to localize antimicrobial activity on a chromatogram. Method involves spotting of extract on TLC plate. Then develop chromatogram in suitable solvent and dry. Spray microbial strain suspension on plate and incubate for required period. Then spray with suitable agent and observe for the antimicrobial activity.

MATERIAL AND METHOD

Preparation and extraction

The leaves of the plants were collected from forrest department office at Amravati. The plant was authenticated at botany department of VMV. The leaves were dried in shade and was powdered, in the soxhlet apparatus it was kept for successive extraction with Pet. Ether, chloroform, methanol and water. A part of powdered leaves were directly place with water to get total aqeous extract.

Evaluation of antimicrobial activity

Preparation of Culture Media.

The mediums used for the growth of bacteria are Nutrient agar medium.

Composition of Nutrient agar medium:

- Beef extract 10 gm
- Peptone 10 gm
- Sodium chloride 5 mg
- Agar 15 gm
- Water to 1000ml

Each material was dissolved with aid of heat in sufficient water and pH was matained upto 8.0- 8.4 with 5M sodium hydroxide and boiled for 10 minutes. Material was filtered and sterilized at 115^{O}C for 30 minutes and pH was adjusted to 7.3 ± 0.1.

A) Sub-culture (Preparation of seeded broth)

The stains of gram-positive and gram-negative organism were obtained from National Chemical Laboratory, Pune and inoculated in conical flasks containing 100 ml sterile nutrient broth. These conical flasks were incubated at 37^{O}C±1^{O}C for 24 hours. This was referred to as seeded broth or 24 hrs bacterial cultures.

B) Standardization of seeded broth (viable count)

a) Dilution: -By using 1ml micropipette, the 24 hrs-seeded broth of each strain was added to 99 ml of sterile nutrient broth. Seven test tubes containing 9 ml of sterile nutrient broth was prepared and to the first test tube 1ml was added from the above 100 ml sterile nutrient broth leading to the concentration of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} concentrations dilutions of seeded broth were obtained.

b) Inoculation onto nutrient agar Petri dishes: The dilutions were studied by inoculating 0.2 ml of each dilution onto solidified nutrient agar medium by spread plate method. After incubation at 37^{0}C ±1^{0}C for 24 hours the numbers of well-formed colonies on the plate were counted. The seeded broth was then suitably diluted to contain between 10^{-6}-10^{-7} microorganisms of cfu/ml. This was designated as the working stock, which was used for antibacterial studies.

C) Preparation of solution of test compounds: -The solution of the test compound was prepared at the concentration of 20mg/ml by dissolving in dimehyl sulphoxide in stoppered specific gravity bottle and stored in refrigerator. The solution was removed from the refrigerator one-hour prior to use and was allowed to warm up to room temperature. DMSO was used as solvent control.

D) Determination of Antimicrobial Activity by Well diffusion Method (zone of inhibition): - This method depends on the diffusion of the various extracts from a cavity through the solidified agar layer of petri dish, to an extent such that growth of the added microorganism is prevented entirely in circular area or zone around the cavity containing the extracts. Using micropipette, 0.2ml of each of the seeded broth containing 10^{-6} –10^{-7}-cfu/ml test organisms was inoculated on the two plates of solidified agar and spreaded uniformly with a glass spreader. Then 3 wells were cut out in the agar layer of each plate with an aluminum bore of 6mm diameter. To the five wells the solution of five extracts (20mg/ml) in DMSO each 0.2 ml was added and the sixth well was kept as solvent control adding only 0.2ml of DMSO and 2mg/ml concentration of tetracycline was used as standard. All the work was carried out under strict aseptic conditions. The plates were kept in freeze for 30 min. after addition to allow diffusion of the solution into the medium and then incubated at 37^{O}C ±1 for 18 hrs. After the incubation period the mean diameter of the zone of inhibition in mm obtained around the well was measured which has been shown in Table.

Anti-fungal study

Anti-fungal study was carried out through same procedure as used in antibacterial study only difference was media used for antifungal study was Sarboud's dextrose agar media (SDA MEDIUM).

Composition of SDA medium.

Mycological peptone	10 gm
Dextrose	40 gm
Agar	15 gm

All ingredients were dissolved in required water with aid of heat. Medium was then sterilized by autoclaving for 15 min. And pH was adjusted to 5.4.

Determination of minimum inhibitory concentration

(By two fold serial dilution method)

The test was carried out individually for different microorganisms with respect to the different solvent extract. This testing was done in the seeded broth by two-fold serial dilution technique. The solution of different extracts was prepared of concentration 20mg in1ml DMSO and a control containing only DMSO. Similarly the standard drug concentration 2mg/ml of Tetracycline was prepared. For both Gram positive and Gram-negative organism a

series of 7 assay tubes for each extract, 1 positive control tube and negative control was prepared. To each test tube the plant extract concentration 20,10,5,2.5,1.25,0.625, 0.3125mg/ml were used for the assay. To all, 0.1ml of suspension of bacteria was added and the tubes were incubated at 37C for 24 hours. The growth in the tubes was observed visually for turbidity and inhibition was determined for absence of growth .MIC was determined by the lowest concentration of sample that prevented the development of turbidity. Determination of Minimum inhibitory concentration (MIC).

(By two fold serial dilution method)

The similar procedure was adopted for the antifungal studies as followed for antibacterial study. But for antifungal study gentamycin (2mg/ml) was used as a standard drug and the temperature was maintained at 28°C 1 for 24 hrs. The MIC study of observation of antifungal studies are shown in above table.

RESULT AND DISCUSSION

Antibacterial activity of Various Extract *of Soymida febrifuga* A. Juss.

A) By well diffusion method.

Table no.18:PET. ETHER EXTRACT

Sr.No.	Microorganisms	Strain Type	Zone of Inhibition of Pet ether Extract(mm)				
A)Gram positive strain			I set	II set	III set	MEAN	Std.Dev.
1	*B. subtillis*	NCIM-2063	12	15	17	14.66	2.05
2	*S. aureus*	NCIM-2608	14	11	16	13.66	2.51
B)Gram negative strain							
1	*E. coli*	NCIM-2065	17	16	18	17	1
2	*P. vulgaris*	NCIM 2813	12	11	15	12.66	2.08
3	*P.aeurginosa*	NCIM 2036	16	19	15	16.66	2.08

Table no. 19: CHLOROFORM EXTRACT

Sr.No.	Microorganisms	Strain Type	Zone of Inhibition of Chloroform Extract(mm)				
A)Gram positive strain			**I set**	**II set**	**III set**	**MEAN**	**Std. Dev.**
1	*B. subtillis*	NCIM-2063	12	17	17	15.33	2.35
2	*S. aureus*	NCIM-2608	14	16	17	15.66	1.52
B)Gram negative strain							
1	*E. coli*	NCIM-2065	13	15	17	15	2
2	*P. vulgaris*	NCIM 2813	15	14	18	15.66	2.08
3	*P. aeurginosa*	NCIM 2036	17	18	17	17.33	0.57

Table no. 20: METHANOL EXTRACT

Sr.No.	Microorganisms	Strain Type	Zone of Inhibition of Methanolic

			Extract(mm)				
A)Gram positive strain			I set	II set	III set	MEAN	Std. Dev.
1	*B. subtillis*	NCIM-2063	18	19	17	18	1
2	*S. aureus*	NCIM-2608	18	17	20	18.33	1.52
B)Gram negative strain							
1	*E. coli*	NCIM-2065	18	17	17	17.33	0.57
2	*P. vulgaris*	NCIM 2813	18	17	19	18	1
3	*P. aeurginosa*	NCIM 2036	19	17	19	18.33	1.15

Table no. 21: WATER EXTRACT

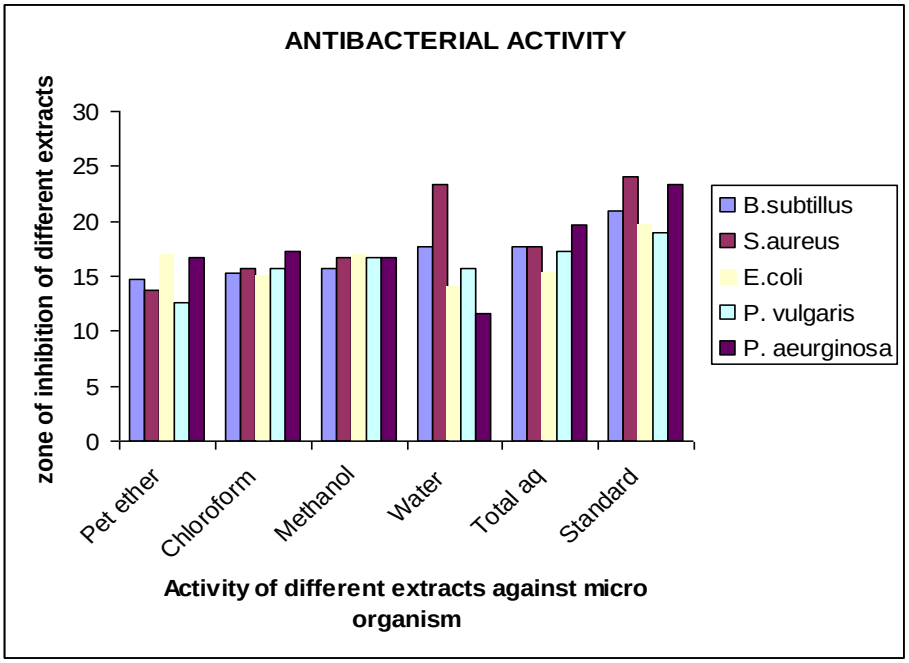
Sr.No.	Microorganisms	Strain Type	Zone of Inhibition of Water Extract(mm)				
A)Gram positive strain			I set	II set	III set	MEAN	Std. Dev.
1	*B. subtillis*	NCIM-2063	17	18	18	17.66	0.57
2	*S. aureus*	NCIM-2608	24	25	21	23.33	2.08
B)Gram negative strain							
1	*E. coli*	NCIM-2065	11	16	15	14	2.64
2	*P. vulgaris*	NCIM 2813	16	15	16	15.66	0.57
3	*P. aeurginosa*	NCIM 2036	13	12	10	11.66	1.52

Table no. 22: TOTAL AQUEOUS EXTRACT

Sr.No.	Microorganisms	Strain Type	Zone of Inhibition of Total aqueous Extract(mm)				
A)Gram positive strain			I set	II set	III set	MEAN	Std. Dev.
1	*B. subtillis*	NCIM-2063	16	17	20	17.66	2.08
2	*S. aureus*	NCIM-2608	17	20	16	17.66	2.08
B)Gram negative strain							
1	*E. coli*	NCIM-2065	12	18	16	15.33	3.05
2	*P. vulgaris*	NCIM 2813	18	18	16	17.33	1.15
3	*P. aeurginosa*	NCIM 2036	20	21	18	19.66	1.52

Table no. 23: STANDARD (TETRACYCLINE 2mg/ml)

Sr.No.	Microorganisms	Strain Type	Zone of Inhibition of std (mm)				
A)Gram positive strain			I set	II set	III set	MEAN	Std. Dev.
1	*B. subtillis*	NCIM-2063	21	22	20	21	1
2	*S. aureus*	NCIM-2608	23	24	25	24	1
B)Gram negative strain							
1	*E. coli*	NCIM-2065	20	19	20	19.66	0.57
2	*P. vulgaris*	NCIM 2813	19	19	19	19	0
3	*P. aeurginosa*	NCIM 2036	23	23	24	23.33	0.57



Graph no.10: Showing antibacterial activity of *Soymida febrifuga* A. Juss.

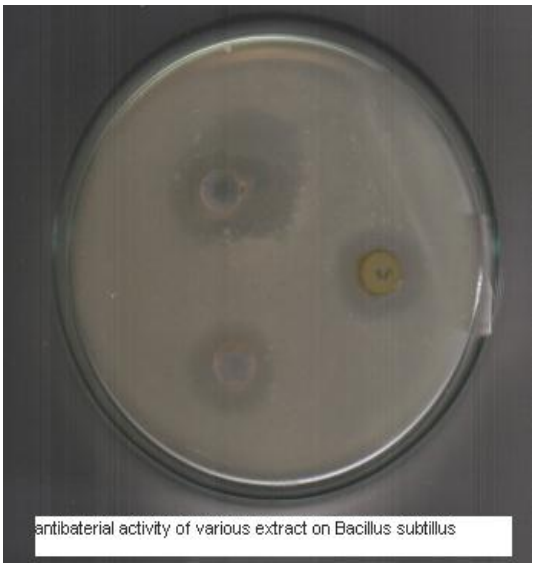


Fig. no.2

Fig. no.3

An appropriate zone of inhibition was recorded from this study so the further study was carried out for determination of minimum inhibitory concentration.

B)

Data showing MIC of different extract by two-fold serial dilution method.

Table no.24: Inhibitory concentration of methanolic extract of *Soymida febrifuga* A. Juss on different microorganism

Sr. No	MICROORGANISM	CONCENTRATION (mg/ml)						MIC (mg/ml)
		20	10	5	2.5	1.25	0.625	
1	*P.aeurginosa*	-	-	-	-	-	+	1.25
2	*B. subtillus*	-	-	+	++	+++	++++	10
3	*E. coli*	-	-	-	+	++	+++	5
4	*P. vulgaris*	-	-	-	-	-	+	1.25

+ indicates turbidity and – indicates no turbidity

Table no.25: Inhibitory concentration of water extract of *Soymida febrifuga* A. Juss on different microorganism

Sr.NO	MICROORGANISM	CONCENTRATION (mg/ml)						MIC(mg/ml)
		20	10	5	2.5	1.25	0.625	
1	*P.aeurginosa*	-	-	-	+	++	+++	1.25
2	*B. subtillus*	-	-	-	-	+	+ +	10
3	*E. coli*	-	-	-	-	-	+	5
4	*P. vulgaris*	-	-	-	+	++	+++	1.25

+ indicates turbidity and – indicates no turbidity

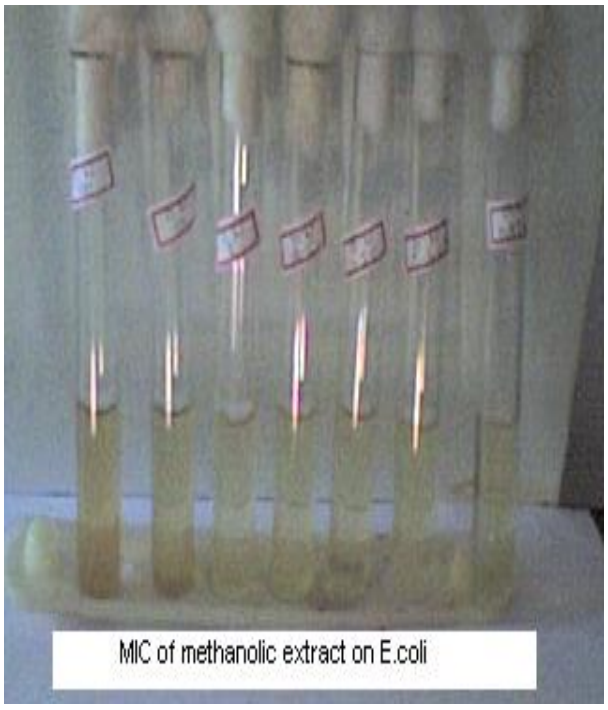


Fig. no. 4

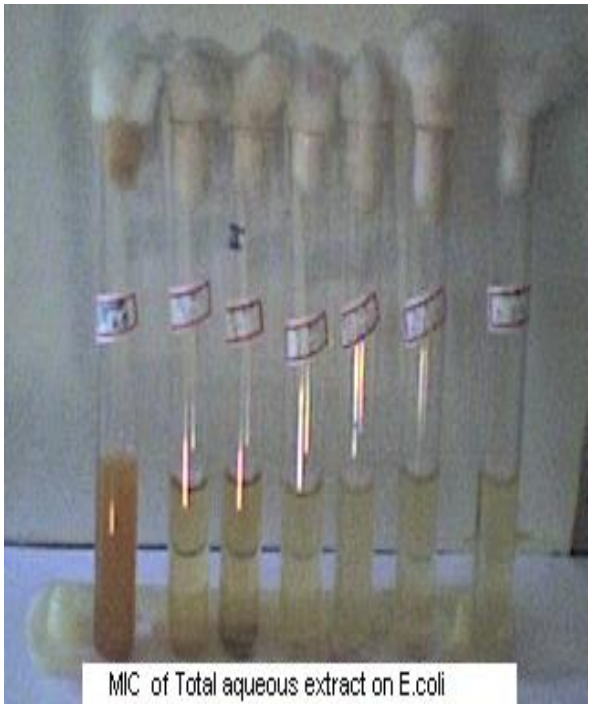
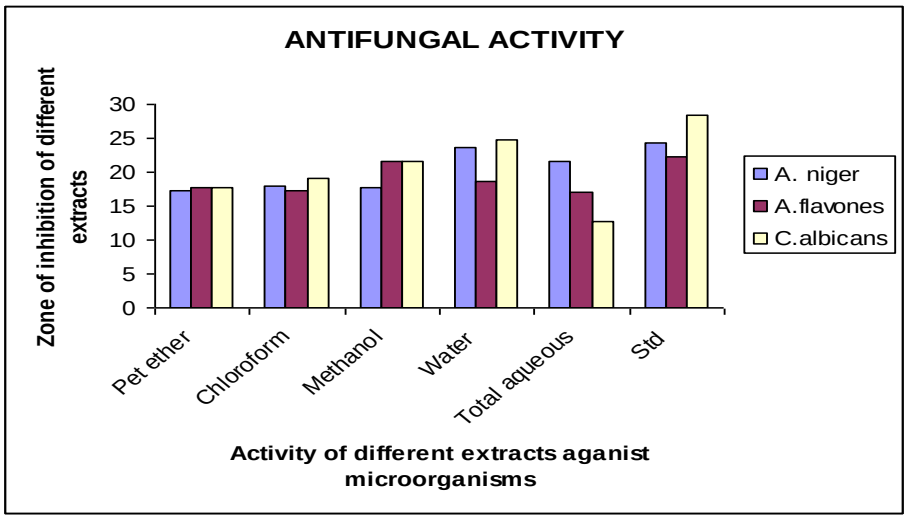


Fig. no.5

A) Agar well diffusion method

Table No. 26: Data showing anti-fungal activity of different extracts by Agar well diffusion method

Sr.No.	Microorganisms	Zone of Inhibition of Pet ether extract(mm)				
	Type of fungi's	I set	IInd set	III set	MEAN	Std.Dev.
1	*Aspergillus Niger*	17	18	17	17.33	0.57
2	*Aspergillus Flavones*	18	18	17	17.66	0.57
3	*Candida albicans*	17	18	18	17.66	0.57
Sr.No.	**Microorganisms**	**Zone of Inhibition of Chloroform extract (mm)**				
	Type of fungi's	I st set	IInd set	III set	MEAN	Std.Dev.
1	*Aspergillus Niger*	16	19	19	18	1.732
2	*Aspergillus Flavones*	16	18	18	17.33	1.154
3	*Candida albicans*	18	20	19	19	1
Sr.No.	**Microorganisms**	**Zone of Inhibition of Methanol extract (mm)**				
	Type of fungi's	Ist set	IInd set	III set	MEAN	Std.Dev.
1	*Aspergillus Niger*	16	19	18	17.66	1.52
2	*Aspergillus Flavones*	18	24	23	21.66	3.21
3	*Candida albicans*	22	21	22	21.66	0.57
Sr.No.	**Microorganisms**	**Zone of Inhibition of Water extract (mm)**				
	Type of fungi's	I st set	IInd set	III set	MEAN	Std.Dev.
1	*Aspergillus Niger*	24	24	23	23.66	0.57
2	*Aspergillus Flavones*	22	13	21	18.66	4.93
3	*Candida albicans*	24	26	24	24.66	1.15
Sr.No.	**Microorganisms**	**Zone of Inhibition of Total Aqueous extract (mm)**				
	Type of fungi's	I st set	IInd set	III set	MEAN	Std.Dev.
1	*Aspergillus Niger*	14	26	25	21.66	6.65
2	*Aspergillus Flavones*	16	17	18	17	1
3	*Candida albicans*	12	13	13	12.66	0.57
Sr.No.	**Microorganisms**	**Zone of Inhibition of Standard (Gentamycin 2mg/ml)(mm)**				
	Type of fungi's	I st set	IInd set	III set	MEAN	Std.Dev.
1	*Aspergillus Niger*	18	28	27	24.33	5.50
2	*Aspergillus Flavones*	18	24	25	22.33	3.78
3	*Candida albicans*	28	30	27	28.33	1.52



Graph No.11: showing anti-fungal activity of different extracts

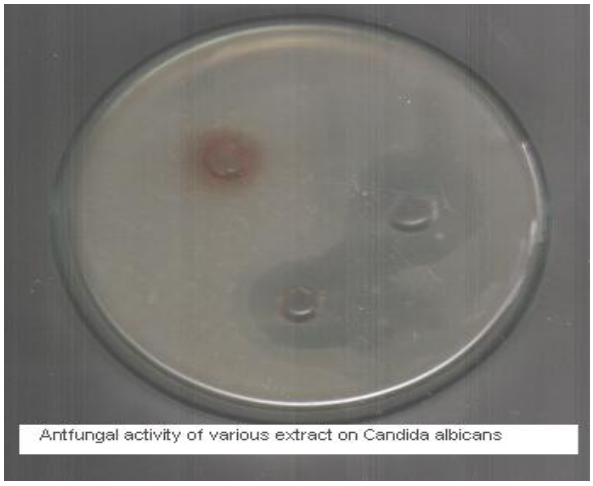


Fig. no.6

Fig. no.7

Determination of Minimum inhibitory concentration (MIC)

Table no.27: Inhibitory concentration of Methanolic extract of *Soymida febrifuga* A. Juss on different microorganism

Sr. NO	MICROORGANISM	CONCENTRATION (mg/ml)						MIC(mg/ml)
		20	10	5	2.5	1.25	0.625	
1	*P.aeurginosa*	-	-	-	-	+	++	2.5
2	*C. albicans*	-	-	-	-	-	+	1.25

+ indicates turbidity and – indicates no turbidity

Table no.28: Inhibitory concentration of water extracts of *Soymida febrifuga* A. Juss on different microorganism.

Sr. No.	MICROORGANISM	CONCENTRATION (mg/ml)						MIC(mg/ml)
		20	10	5	2.5	1.25	0.625	
1	*P.aeurginosa*	-	-	-	-	-	+	1.25
2	*C. albicans*	-	-	-	-	-	+	1.25

+ indicates turbidity and – indicates no turbidity

Fig. no.8

Fig. no.9

CONCLUSION

According to Ethno medicinal claim *Soymida febrifuga* used in fever, cough and vaginal infections skin diseases therefore to rationalize the claims antimicrobial activity including Gram positive, Gram negative bacteria and some fungal strains have been performed .Study indicates that *Bacillus subtillis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeurginosa, Proteous vulgaris* were used in antibacterial testing .for the extracts of 20mg/ml and Tetracycline 2mg/ml in Dimethyl sulphoxide was used as standard. The minimum inhibitory concentration of each extract was determined by reported procedure.

Candida albicans, Aspergillus Niger and *Aspergillus flavones* were used in antifungal testing. For it also 20mg/ml extracts and gentamycine 2mg /ml in DMSO were used as standard. All five extracts exhibited prominent antimicrobial activity against all microorganisms used in the study. From the zone of inhibition (Table No. 17, 18, 19, 20, 21) produced by the extracts, it is observed that methanol, water and total aqueous extracts showing prominent antimicrobial activity against all microorganisms.

Table.22, 24. Gives the average value of MIC in mg/ml of various extracts. Methanol extract shows prominent activity against *Pseudomonas aeurginosa*. (MIC 0.625mg/ml), *Bacillus subtillis* (MIC 5mg/ml) and *Candida Albicans* (MIC 0.625mg/ml); while water extract against *Proteous vulgaris*. (MIC 2.5 mg/ml).

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