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Research Article

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Girnar Mountain Forest Soil near Herbal Plant Area Screening Study of Atinomycetes for Antimicrobial Activity with Characterization of Active Isolates

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ABSTRACT

Objective: *Present study screening of soil actinomycetes near herbal plant area in Girnal mountain region of Junagadh district of Saurashtra Gujarat in respect to its antimicrobial activity and biochemical characterization of active isolates.* ***Methods:*** *11 different herbal plant sites selected randomly from Girnar mountain area. Soil collected from microflora part of soil depth up to 30 cm with sterile articles used. Samples treated and serially diluted for good colony result. Different growth and ISP media tested for colony study and make pure culture using microbial method. Morphological and microscopic study was done of colonies. Antimicrobial activity checked by cross streak method tested against various test pathogens. Secondary screening was done by agar well diffusion method. Various biochemical testes for characterization were done of active isolates.* ***Results:*** *23 isolates of actinomycetes were observed with dominant arial mycelium white color in 56.52% of isolates and 43.48% yellow color in vegetative mycelium. 13 (56.52%) isolates were classified and belongs to streptomyces genus. More than 20 mm ZOI (Zone of inhibition) observed in 3 isolates (against Bacillus subtilis), 4 isolates (against Staphylococcus aureus), 2 isolates (against Proteus vulgaris), 2 isolates (against Escherichia coli) and 2 isolates (against Klebsiella aerogenes). JRI1, JOS1, JAC1, JTF1 and JPC1 were observed most active isolates.* ***Conclusion:*** *Out of active isolates range of ZOI was between 15 to 20 mm is most frequently observed. 11 isolates showed activity and 12 isolates not showed any activity against test pathogens. Soil actinomycetes near some medicinal plant area of Girnar forest sites contain actinomycetes which have antagonistic activity. As Girnar forest was very rich source of herbal medicinal plants we correlate soil actinomycetes screening study for antimicrobial activity samples taken near plant area and these ecological sites had potential to produce new antimicrobial compound with other metabolites from those actinomycetes.*

Keywords: Actinomycets; Mycelium; Antimicrobial; Isolate; Medicinal

Abbreviations

ZOI: Zone of Inhibition, Ec: *Escherichia coli*, Sa: *Staphylococcus aureus*, Pa: *Pseudomonas aeruginosa*, Et: *Enterobacter aerogenes*, Pv: *Proteus vulgaris*, St: *Salmonella typhi*, Bs: *Bacillus subtilis*, Ka: *Klebsiella aerogenes*

INTRODUCTION

Girnar mountain height at 2672 ft is one of the oldest hills in Gujarat with most diversified forest area covering contains so many numbers of medicinal plants. Amongst those plants many have antimicrobial activity. Soil contain many types of microorganisms amongst them actinomycetes are most important in respect to study of many bioactive compounds like antibiotics, metabolites and other industrially important compounds from them.

Actinomycetes are gram +ve in nature freely living or found in various natural sources like soil parts of various places and water also contains large amount of actinomycetes. Around 50% of compounds have biological activity like antibiotics are produced from them followed by fungi, unicellular bacteria and other sources [1].

There are many species observed in actinomycetes amongst them streptomyces genus is contain larger proportion in it. And very useful in production of many important compounds like antibiotics, anticancer drugs, antiviral drugs, enzymes and many biological secondary metabolites [2,3]. Soil is very rich source of microbes. Present study we screened soil near different medicinal plant area of Girnar forest site of junagadh. Study actinomycetes observed from those sites.

More than 50% of antibiotics produced from streptomyces species of actinomycetes contain higher G and C contents, positive in grams staining [4,5] also today many of the microorganism developed resistance against various available medicines and the requirement of new compound having antimicrobial activity are very necessary for future of healthcare. Hence to screen unexplored ecological area is one of the ways to do so [6,7].

Screening and isolation study of sites like Girnar forest area having rich in its medicinal plants collection and diversified ecological places may be useful to this aim. Many research also done having states that soil actinomycetes having capability to produce novel antimicrobial compounds.

Objective of present study is screening of soil actinomycetes near herbal plant area in Girnal mountain region of Junagadh district of Saurashtra Gujarat in respect to its antimicrobial activity and biochemical characterization of active isolates.

MATERIALS AND METHODS

Sample Collection

11 different medicinal plants Aloe vera, *Azadirachta indica*, *Syzygium cumini*, *Datura stramonium*, *Rosa indica*, *Pongamia pinnata*, *Oscimum sanctum*, *Allium sativum*, *Allium cepa*, *Trigonella foenum*-graecum and *Psoralea corylifolia* selected on the bases of some part of those plants having antimicrobial activity from Girnar mountain forest region of junagadh Gujarat. Soil samples were collected from near soil of those medicinal plants within 30 cm depth area of microflora part of soil with help of sterile articles and and treat soil samples air dried and stores at 4°C into refrigerator until use.

Sample Preparation

Dilution methods were used to produce visible and dusting colony of actinomycetes. In 99 ml of sterile water add one gram of dried soil and make serial dilution by adding sterile water make dilution 4 times to get 10-5 samples vortex soil water mixture every time during dilution. 0.1 ml of aliquot prepared from this dilution method [8].

Different growth media were prepared and sterile Starch casein digest agar media, nutrient agar media, and actinomycetes agar media with previously added antifungal agent Nystatin 50 ug/ml in all media preparations. Agar plates were prepared under aseptic conditions and incubated at 35°C. 1 g of dried soil sample was taken and add in to flask contain 9 ml of sterile water and make consecutive steps by transferring 1 ml from each step and added into second step of sample preparation. By this serially steps of dilution make 10−5 sample. Mixture was vortex continuously evertime and make uniform suspension. Sample 0.1 ml used and spread on the surface of growth media previously added with nystatin (50 µg/mL) on petri dish then incubated at °C for 7 to 8 days [9-13].

Colony forming unit per gram was determined and plates having nearby 150 colonies taken for further process to making pure colony of actinomycetes. Pure culture of actinomycetes was stored by using same media in which they developed maximum growth by adding glycerol 15% (v/v) for longer preservation of culture for future use at -20°C [14].

Morphological and Microscopic Study

Colony morphology was studied in respect to color of arial mycelium and reverse side mycelium with characteristics of soil samples [15]. Microscopic examination was done to identify and study morphological charecters of actinomycetes isolates up to genus level by electron microscopy.

Screening of Isolates

Initial primary level screening was done by using cross streak method on MHA media. Growth media was prepared and sterile by autoclave at 121°C for 30 min. and make agar plates under aseptic cabinet under precautions to minimize contamination. Incubate at 35°C for 7 to 8 days duration.

Test organism were streaked on straight line in plates with growth of microbes in agar plates towards middle line of microbial growth and incubated under defined conditions then observed zone of inhibition in plates [16].

By this study identified to most 5 isoltaes having maximum zone of inhibition and used for secondary screening study of those isolates by agar well diffusion method. Well with 7 to 8 mm was prepared on media and add 20 to 100 µl of microbial suspension on this and incubate at define conditions for growth.

Biochemical Analysis

Characteristics of active actinomycetes isolates were studied by observed growth in various ISP media, staining of microbes, utilization of different sugars by isolates, Voges-Proskauer (VP) Test, Hydrogen Sulphide Production test, Methyl red Test, Citrate test and Indol Test of most active 5 isolates [17].

RESULTS AND DISCUSSION

Figure 1 indicates diluted soil samples growth on starch casein agar media. By using 11 different medicinal plant soil in Girnar mountain forest area of Junagadh.

Figure 1. Growth of microbes on Starch casein agar media, Sample*:* Near *Oscimum sanctum* plant soil, Place: Junagadh, Dilutions: From left to right 10^{-2}, 10^{-3}, 10^{-4}

From 11 sites total 23 actinomycetes isolates observed based on their morphological parameters as shown in Table 1.

Table 1. Growth of actinomycetes from different sites samples with characteristics

Soil near plant	No. of isolate	Characters	Aerial mycelium color	Vegitative mycelium color	Code
Aloe vera	1	Rough	White	White	JAV1
	2	Rough	Yellow	Yellow	JAV2
	3	Leathery	Cream	Cream	JAV3
Azadirachta indica	4	Rough	White	Yellow	JAI1
	5	Powdery	White	Brown	JAI2
	6	Wrinkled	Yellow	Gray	JAI3
Syzygium cumini	7	Rough	White	Yellow	JSC1
	8	Powdery	Cream	White	JSC2
Datura stramonium	9	Rough	White	White	JDS1
	10	Rough	White	Yellow	JDS2
Rosa indica	11	Rough	White	Yellow	JRI1
	12	Powdery	White	Yellow	JRI2
	13	Smooth	Gray	Gray	JRI3
Pongamia pinnata	14	Smooth	Cream	White	JPP1
	15	Smooth	White	Yellow	JPP2
Oscimum sanctum	16	Rough	White	Yellow	JOS1
	17	Wrinkled	Gray	Gray	JOS3
Allium sativum	18	Rough	White	White	JAS1
	19	Wrinkled	Brown	Yellow	JAS2
Allium cepa	20	Rough	Brown	Brown	JAC1
Trigonella foenum-	21	Rough	White	Yellow	JTF1

graecum	22	Cottony	White	White	JTF2
Psoralea corylifolia	23	Rough	Brown	Brown	JPC1

It was observed that out of 23 isolates 13 showed white color in arial mycelium, 2 showed yellow color, 3 showed cream color, 2 showed gray color and 3 showed brown color in arial mycelium of actinomycetes colonies. So here dominant color was white while in vegetative mycelium color it was observed that white in 6 isolates, yellow in 10 isolates, cream in 1 isolate, gray in 3 isolates and brown in 3 actinomycetes isolates. So here dominant color was yellow.

By electron microscopy examination of all 23 actinomycetes isolates identification was microbes up to genus level was done according bergey's manual of systematic bacteriology. Various parameters studied like long chains of spores present on aerial mycelium, vegetative hyphae produce branched mycelium, single condidia formed on subsrate mycelium in large black mucoid masses, only substrate mycelium formed terminal, subterminal vesicals, vegitative mycelium is developed, aerial mycelium straight or spiral segmented chains of spores, bread like chains of spores, aerial mycelium formed globose spore vesicles, rodes to extensively branched mycelium may be found, produce predominantly single spore on aerial mycelium and classified on genus level of isolates as shown in Figure 2.

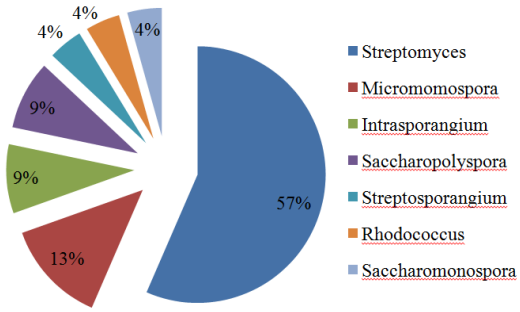

Figure 2. Percentage distribution of actinomycete isolates based on their genus

It was found that 57% of actinomycetes isolates belongs to streptomyces genus, 13% actinomycetes isolates belongs to micromonospora genus, 9% actinomycetes isolates belongs to intrasporangium genus, saccharopolyspora genus, and 4% actinomycetes isolates belongs to Streptosporangium genus, Rhodococcus genus and Saccharomonospora genus. Out of all isolates more than 50% observed under streptomyces genus and found in soil collected from different medicinal plant area of Girnar mountain forest sites.

Primary Screening of Isolates

In cross streak method used for primary screening it was observed that soil samples obtained from near medicinal plant Syzygium cumini, Datura stramonium and Pongamia pinnata produce actinomycetes note showed any activity against 8 test pathogens. While soil near medicinal plant are of Aloe vera, Azadirachta indica, Rosa indica, Oscimum sanctum, Allium sativum, Allium cepa, Trigonella foenum-graecum and Psoralea corylifolia showed activity against test organisms. Table 2 showed values of three replicate agar plates in mm zone of inhibition.

Table 2. Inhibition in mm of test Actinomycetes isolates against various pathogens

Microbial species/3 relicate		Actinomycetes isolates										
		JAV1	**JAV2**	**JAI1**	**JRI1**	**JRI2**	**JOS1**	**JOS3**	**JAS1**	**JAC1**	**JTF1**	**JPC1**
Bs	Mean	20.33	18	18.33	22	16.33	22.33	4.33	18	18.33	22.33	18
	± SD	1.53	1	1.53	1.73	1.15	0.58	1.53	0	0.58	1.53	1
Sa	Mean	19.67	21	17	23.33	17.33	21	4.33	18.67	0	22.67	16
	± SD	1.15	1	0	1.53	0.58	0	0.58	1.53	0	1.53	1
Pv	Mean	18.67	17.67	0	0	0	23	0	17.33	19.67	22	16.33
	± SD	1.15	1.53	0	0	0	1	0	0.58	1.53	1	0.58
Ec	Mean	19.67	17.33	20	19	17.67	22.67	3.33	17.33	17.33	23	18.67
	± SD	0.58	1.53	1	1	0.58	0.58	0.58	0.58	1.15	1	1.53
Ka	Mean	14.33	19.33	18.67	18.67	18	24.33	0	17.33	18.33	24.33	16.67
	± SD	0.58	0.58	1.53	1.53	1	0.58	0	0.58	0.58	0.58	0.58
Pa	Mean	0	0	20.33	19.33	16.33	0	0	16.33	0	20	16.33
	± SD	0	0	2.08	1.15	0.58	0	0	0.58	0	1	0.58
St	Mean	4	0	0	0	0	19	2.33	0	18.33	0	16.67
	± SD	1	0	0	0	0	1	1.15	0	1.53	0	1.15
Et	Mean	0	0	0	16.67	17.33	17.67	0	0	20	0	0
	± SD	0	0	0	0.58	0.58	0.58	0	0	1	0	0

Against *Bacillus subtilis* JAV1 showed 20.33 ± 1.53 mm ZOI, JAV2 showed 18.00 ± 1.00 mm ZOI, JAI1 showed 18.33 ± 1.53 mm ZOI, JRI1 showed 22.00 ± 1.73 mm ZOI, JRI2 showed 16.33 ± 1.15 mm ZOI, JOS1 showed 22.33 ± 0.58 mm ZOI, JOS3 showed 4.33 ± 1.53 mm ZOI, JAS1 showed 18.00 ± 0.00 mm ZOI, JAC1 showed 18.33 ± 0.58 mm ZOI, JTF1 showed 22.33 ± 1.53 mm ZOI and JPC1 showed 18.00 ± 1.00 mm ZOI. Against *Staphylococcus aureus* JAV1 showed 19.67 ± 1.15 mm ZOI, JAV2 showed 21.00 ± 1.00 mm ZOI, JAI1 showed 17.00 ± 0.00 mm ZOI, JRI1 showed 23.33 ± 1.53 mm ZOI, JRI2 showed 17.33 ± 0.58 mm ZOI, JOS1 showed 21.00 ± 0.00 mm ZOI, JOS3 showed 4.33 ± 0.58 mm ZOI, JAS1 showed 18.67 ± 1.53 mm ZOI, JAC1 showed 0.00 ± 0.00 mm ZOI, JTF1 showed 22.67 ± 1.53 mm ZOI and JPC1 showed 16.00 ± 1.00 mm ZOI.

Against *Proteus vulgaris* JAV1 showed 18.67 ± 1.15 mm ZOI, JAV2 showed 17.67 ± 1.53 mm ZOI, JAI1 showed 00.00 ± 0.00 mm ZOI, JRI1 showed 0.00 ± 0.00 mm ZOI, JRI2 showed 0.00 ± 0.00 mm ZOI, JOS1 showed 23.00 ± 1.00 mm ZOI, JOS3 showed 0.00 ± 0.00 mm ZOI, JAS1 showed 17.33 ± 1.58 mm ZOI, JAC1 showed 19.67 ± 1.53 mm ZOI, JTF1 showed 22.00 ± 1.00 mm ZOI and JPC1 showed 16.33 ± 0.58 mm ZOI.

Against *Escherichia coli* JAV1 showed 19.67 ± 0.58 mm ZOI, JAV2 showed 17.33 ± 1.53 mm ZOI, JAI1 showed

20.00 ± 1.00 mm ZOI, JRI1 showed 19.00 ± 1.00 mm ZOI, JRI2 showed 17.67 ± 0.58 mm ZOI, JOS1 showed 22.67 ± 0.58 mm ZOI, JOS3 showed 3.33 ± 0.58 mm ZOI, JAS1 showed 17.33 ± 0.58 mm ZOI, JAC1 showed 17.33 ± 1.15 mm ZOI, JTF1 showed 23.00 ± 1.00 mm ZOI and JPC1 showed 18.67 ± 1.53 mm ZOI. Against *Klebsiella aerogenes* JAV1 showed 14.33 ± 0.58 mm ZOI, JAV2 showed 19.33 ± 0.58 mm ZOI, JAI1 showed 18.67 ± 1.53 mm ZOI, JRI1 showed 18.67 ± 1.53 mm ZOI, JRI2 showed 18.00 ± 1.00 mm ZOI, JOS1 showed 24.33 ± 0.58 mm ZOI, JOS3 showed 0.00 ± 0.00 mm ZOI, JAS1 showed 17.33 ± 0.58 mm ZOI, JAC1 showed 18.33 ± 0.58 mm ZOI, JTF1 showed 24.33 ± 0.58 mm ZOI and JPC1 showed 16.67 ± 0.58 mm ZOI.

Against *Pseudomonas aeruginosa* JAV1 showed 0.00 ± 0.00 mm ZOI, JAV2 showed 0.00 ± 0.00 mm ZOI, JAI1 showed 20.33 ± 2.08 mm ZOI, JRI1 showed 19.33 ± 1.15 mm ZOI, JRI2 showed 16.33 ± 0.58 mm ZOI, JOS1 showed 0.00 ± 0.00 mm ZOI, JOS3 showed 0.00 ± 0.00 mm ZOI, JAS1 showed 16.33 ± 0.58 mm ZOI, JAC1 showed 0.00 ± 0.00 mm ZOI, JTF1 showed 20.00 ± 1.00 mm ZOI and JPC1 showed 16.33 ± 0.58 mm ZOI. Against *Salmonella typhi* JAV1 showed 4.00 ± 1.00 mm ZOI, JAV2 showed 0.00 ± 0.00 mm ZOI, JAI1 showed 0.00 ± 0.00 mm ZOI, JRI1 showed 0.00 ± 0.00 mm ZOI, JRI2 showed 0.00 ± 0.00 mm ZOI, JOS1 showed 19.00 ± 1.00 mm ZOI, JOS3 showed 2.33 ± 1.15 mm ZOI, JAS1 showed 0.00 ± 0.00 mm ZOI, JAC1 showed 18.33 ± 1.15 mm ZOI, JTF1 showed 0.00 ± 0.00 mm ZOI and JPC1 showed 16.67 ± 1.15 mm ZOI.

Against *Enterobacter aerogenes* JAV1 showed 0.00 ± 0.00 mm ZOI, JAV2 showed 0.00 ± 0.00 mm ZOI, JAI1 showed 0.00 ± 0.00 mm ZOI, JRI1 showed 16.67 ± 0.58 mm ZOI, JRI2 showed 17.33 ± 0.58 mm ZOI, JOS1 showed 17.67 ± 0.58 mm ZOI, JOS3 showed 0.00 ± 0.00 mm ZOI, JAS1 showed 0.00 ± 0.00 mm ZOI, JAC1 showed 20.00 ± 1.00 mm ZOI, JTF1 showed 0.00 ± 0.00 mm ZOI and JPC1 showed 0.00 ± 0.00 mm ZOI.

As shown in Figure 3 represent that 11 (48%) actinomycetes isolates were shown activity against *Bacillus subtilis* and 12 (52%) not shown any activity, 10 (43%) actinomycetes isolates were shown activity against *Staphylococcus aureus* and 13 (57%) not shown any activity, 7 (30%) actinomycetes isolates were shown activity against *Proteus vulgaris* and 16 (70%) not shown any activity, 11 (48%) actinomycetes isolates were shown activity against *Escherichia coli* and 12 (52%) not shown any activity, 10(43%) actinomycetes isolates were shown activity against *Klebsiella aerogenes* and 13 (57%) not shown any activity, 6(26%) actinomycetes isolates were shown activity against *Pseudomonas aeruginosa* and 17 (74%) not shown any activity, 5(22%) actinomycetes isolates were shown activity against *Salmonella typhi* and 18 (78%) not shown any activity, 4(17%) actinomycetes isolates were shown activity against *Enterobacter aerogenes* and 19 (83%) not shown any activity (Figure 4).

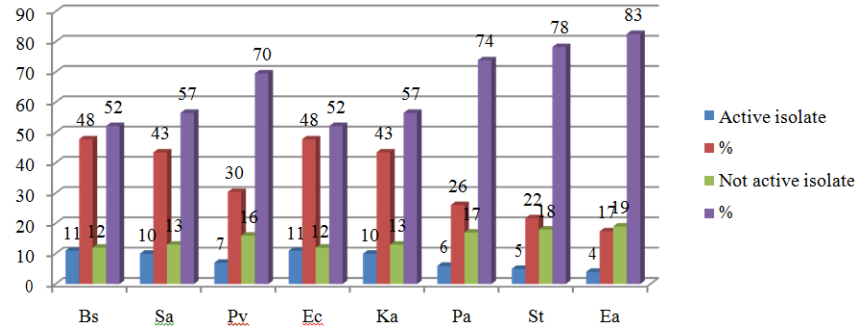

Figure 3. Primary screening distribution of actinomycetes isolate for antimicrobial activity against various test pathogens

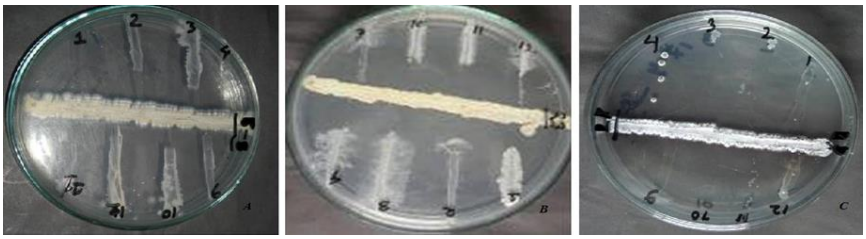

Figure 4. Antimicrobial activity by streak plate method (A) Low active isolate JDS1 (B) moderate active isolate JAC1 (C) highly active isolate JOS1 against various test organisms

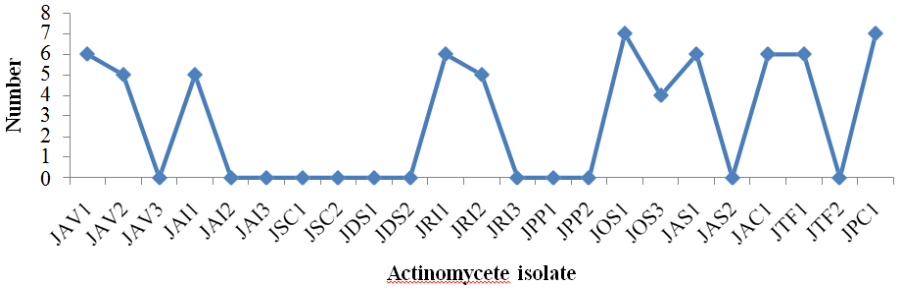

Figure 5. Number distribution of various actinomycetes isolate showed activity against test pathogens

As shown in Figure 5 it was observed that JAI3, JSC1, JSC2, JDS1 and JPP1 isolates not shown any activity against any test microbes. JAV1, JRI1, JAS1, JAC1 and JTF1 showed activity against 6 different test organisms. JOS1 and JPC1 showed activity against 7 different test microbes. JOS3 showed activity against 4 various test pathogens.

Table 3. Percentage distribution of zone of inhibition in various ranges

Sr. No	ZOI (mm)	%	Bs	Sa	Pv	Ec	Ka	Pa	St	Et
1	>20	%	13.04	17.39	8.7	8.7	8.7	0	0	0
2	15 to 20	%	30.43	21.74	21.74	34.78	30.43	26.09	13.04	17.39
3	10 to 15	%	0	0	0	0	4.35	0	0	0
4	<10	%	4.35	4.35	0	4.35	0	0	8.7	0

As shown in Table 3 it was found that more than 20 mm ZOI was observed in 13.04% isolates, against Bs, 17.39%

isolates against Sa, 8.70% isolates against Pv, 8.70% isolates against Ec and Ka, 0% isolates agansit Pa, St and Et. 15 to 20 mm ZOI was observed in 30.43% isolates against Bs, 21.74% isolates against Sa, 21.74% isolates against Pv, 34.78% isolates against Ec, 30.43% isolates against Ka, 26.09% isolates against Pa, 33.04% isolates against St and 17.39% isolates against Et. 10 to 15 mm ZOI was observed 4.35% isolates against Ka, and 0% isolates have shown activity against other test pathogens. Less than 10 mm ZOI 4.35% Isolates against Bs, Sa and Ec while 8.70% isolates shown activity against St and 0% isolates showed activity against rest of isolates. It was found that most activity of isolates found in range of 15 to 20 mm ZOI vs. various test organisms.

Secondary Screening

Based on primary screening data top 5 actinomycetes isolates selected for secondary screening for antimicrobial activity by agar well diffusion method and results were discussed in Table 4.

Table 4. ZOI of active isolates against test organisms by agar well diffusion method

Actinomycetes isolates		Test organisms							
		Bs	**Sa**	**Pv**	**Ec**	**Ka**	**Pa**	**St**	**Et**
JRI1	Mean	21	21.67	0	20	19.33	17	0	18.33
	± SD	1	0.58	0	1	0.58	1	0	0.58
JOS1	Mean	25	19.33	18.67	18.33	20	0	20	21
	± SD	1	0.58	0.58	0.58	1	0	1	1
JAC1	Mean	17.33	2.67	19	18.33	18.33	0	15	16.33
	± SD	0.58	0.58	1	0.58	0.58	0	1	0.58
JTF1	Mean	20.67	22.67	23	20.67	21.33	20	0	0
	± SD	0.58	0.58	1	1.15	0.58	1	0	0
JPC1	Mean	18.33	17.67	16.33	19.33	18.67	17.33	16.67	0
	± SD	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0

Values in Mean ± SD of three replicates.

Secondary screening data also proved that actinomycetes isolates produce from soil near various medicinal plant area of Girnar mountain forest site Junagadh have shown antimicrobial activity against different range of microorganisms.

Biochemical Analysis of Active Actinomycetes Isolates

5 isolates selected from primary screening and analyzed by various biochemical tests like staining of microbes, utilization of different sugars by isolates, Voges-Proskauer (VP) Test, Hydrogen Sulphide Production test, Methyl red Test, Citrate test and Indol Test of most active 5 isolates.

Except JAC1 all 4 actinomycetes were shown gram positive in nature JAC1 was gram negative by gram's staining method.

As shown in Table 5 JRI1 showed good growth in ISP2, ISP3 and actinomycetes agar media, moderate growth in ISP5 and ISP7 media while poor growth was observed in ISP4 media. JOS1 showed good growth in ISP3, ISP5,

actinomycetes agar and ISP7 media, moderate growth was observed in ISP2 media while poor growth was observed in ISp4 media. JAC1 isolate showed good growth in ISP2 media and moderate growth was observed in ISP3, ISP4, ISP5, actinomycetes agar and ISp7 media. JTF1 showed good growth in ISP2, ISP3, ISP5, actinomycete agar and ISp7 media while poor growth was observed in ISp4 media. JPC1 showed good growth in ISP2, ISp3 and actinomycete agar media, moderate growth was observed in ISP5 and ISp7 media, while poor growth was observed in ISp4 media.

Table 5. Growth of actinomycete isolates in various ISP media and biochemical test results

Sr. No	Media	JRI1	JOS1	JAC1	JTF1	JPC1
1	ISP2	+++	++	+++	+++	+++
2	ISP3	+++	+++	++	+++	+++
3	ISp4	+	+	++	+	+
4	ISP5	++	+++	++	+++	++
5	Actinomycetes agar media	+++	+++	++	+++	+++
6	ISP7	++	+++	++	+++	++
+++: Good growth, ++: Moderate growth, +: Poor growth						
Sr. No	**Tests**	**JRI1**	**JOS1**	**JAC1**	**JTF1**	**JPC1**
1	Indol	Positive	Negative	Positive	Negative	Negative
2	Methyl red Test	Negative	Positive	Positive	Positive	Positive
3	Voges-Proskauer (VP) Test	Positive	Negative	Positive	Positive	Negative
4	Citrate test	Negative	Negative	Positive	Positive	Negative
5	Hydrogen Sulphide Production test	Negative	Positive	Negative	Positive	Positive

As shown in Table 5 JRI1 showed positive test result in indol, and VP test and negative in Methyl red Test, Citrate test and Hydrogen Sulphide Production test. JOS1 showed negative test results in indol, VP and citrate test while positive test results in Methyl red Test. JAC1 showed positive test results in indol, Methyl red Test, VP and citrate test while negative result was observed in Hydrogen Sulphide Production test. JTF1 showed negative test result in indol test and positivetest results in Methyl red Test, citrate test, VP test and Hydrogen Sulphide Production test. JPC1 showed positive test result in Methyl red Test and Hydrogen Sulphide Production test while negative test result was shown in indol, VP and citrate test. Various researchers performed related works like study performed [18-20].

CONCLUSION

As Girnar forest was very rich source of herbal medicinal plants we correlate soil actinomycetes screening study for antimicrobial activity in samples taken near medicinal plants having some antimicrobial activity. Aloe vera, Azadirachta indica, Rosa indica, Oscimum sanctum, Allium sativum, Allium cepa, Trigonella foenum-graecum and Psoralea corylifolia medicinal plant area soil was rich source of actinomycetes which contain antimicrobial activity

in Girnar forest mountain region. Some isolates showed more than 20 mm ZOI indicate there are chances to development of new strain and species of actinomycetes which have tendancy to eliminate various range of pathogens that resist against current treatment of medicine. There are great chances to purify most active actinomycetes isolates and compound used in various biological purposes from them like antibiotics, enzymes preparations, secondary metabolites etc. in this respect this ecological regions have capability for that.

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