Study of Antimicrobial Activity of Actinomycetes Isolates from Non-Medicinal Plants Produced Soil & Soil Surrounding Medicinal Plants in Junagadh, India

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Isolation and screening of actinomycetes and compare its antimicrobial activity from non-medicinal plant produced soil with soil surrounding medicinal plant. In this study total 22 soil samples collected in two sets one from soil surrounding medicinal plant area of Junagadh and other not nearby medicinal plant of same region. Primary screening of isolate done using cross streak method with 8 test pathogens. Secondary screening of most potent 10 isolates equal from both sets by agar well diffusion. Thirty two isolate observed from soil samples of 11 places which were not nearby medicinal plants and 36 isolate observed from soil samples collected surrounding 11 different medicinal plants. (A)OS1, (A)OS3, (A)AI1, (A)TF1 and (A)RI1 were most potent isolates produced from soil surrounding medicinal plant compare to set 2. It was found set1 showed 72.22 % and set 2 showed 53.12 % activity.

Keywords: Actinomycets, Potent, Antimicrobial, Isolate, Medicinal.

INTRODUCTION

Soil is most outer layer of surface of earth and its characteristics are different in its layer like most outer layer is loose soil while in deeper layer is hard rock also so many other layers are present in it. For agriculture point of view soil plays important role for development of plants on the surface of earth. In soil there are many biological interactions take places and those are the interest in particularly microbiological aspects. Soil is rich source of diverse microbes. Locality and climate is main factor affecting on microorganism amount and proposition of microorganisms. One of the main functions like decomposition of many organic materials is possible because of the microorganisms. Soil microbiology plays important role in study of microorganisms and their activities [1].

Amongst all the living species on the earth microorganisms is most old in them on our planet. The dependence is in relative manner like we depends on plants for our nutrition. Plants depends on soil for their nutrition and microorganisms are responsible for make soil capable for developing plants. Many researchers studied those microorganisms from various sources like air, water and soil. After that they came to know

that the importance of those microbes in cycle of earth's existence. Before, we came able to know this microorganism exists with their all functions [2]. Also microorganisms decompose the organic material like dead body of species by many biochemical reactions on them so in soil and also many variety of those microorganisms' are present in soil. Ultimately soil is very rich source of diverse microbes.

Mainly soil is composed of organic, mineral, air in soil, water in soil and microorganisms in soil. Locality and climate are main factors affecting on microorganism amount and proposition of microorganisms. Soil is like one living system for different species of microbes. In respect to proportion bacteria is more in numbers while compare with other groups. Because of size of microorganisms they occupy very less space on soil and few percentage. In soil each and every microbes and there group have their specific role like it affects the growth and development of various plants also make soil fertile. Some constitute from animals and plants play as one of components in soil structure. So, soil is one type of medium for all that natures' activities [3].

Soil is cultivated has more rich numbers of microorganisms and other living organisms. Fertility of soil depends on

microbes, which are present in soil and productivity of soil is depending on them. In respect to nutritional sources of environment for maintaining nature's cycle is affected by presence of microorganisms. Some microorganisms are responsible for many human diseases as well as plant and other species diseases. So, both the type of effect is there of microorganism positive and negative.

There are wide application and scope of soil microbiology like pharmaceutical, industrial, medicinal, beverages and spirit production, used in fermented food industries, production of insecticides pesticides, production of amino acids, manufacture of antibiotics, manufacture of therapeutic enzymes, steroids production, single cell protein production, geochemical field, aero microbiology, waste treatment area, agriculture sector, *etc.* Streptomyces is most important amongst all genres of actinomycetes and the genera that are in non-streptomyces group are very rare approximately hundred genera [4-8].

EXPERIMENTAL

All cultural media, chemicals, reagents, diagnostic kit used are from Himedia laboratories, Mumbai, India.

In present study, we selected random places in Girnar forest area of Saurashtra, Gujarat state, India. Soil samples collected in two sets one was soil surrounding *Aloe vera*, *Azadirachta indica*, *Syzygium cumini*, *Datura stramonium*, *Rosa indica*, *Pongamia pinnata*, *Oscimum sanctum*, *Allium sativum*, *Allium cepa*, *Trigonella foenum-graecum*, *Psoralea corylifolia* and another set was non-medicinal plant area soil. Micro flora part of soil is used because maximum numbers of microbes were present in this part of soil. 3 cm surface soil separated and up to 25 cm depth part of soil is used for sample collection. Sterile tube used for collection and storage of samples. Stainless steel sterile spatula and scoops used in collection process.

Sample treatment: During collection, transportation and storage of soil samples, care has been taken to minimize contamination. Air tight sterile tubes were used for storage. In laminar air flow (LAF) soil samples were dried at room temperature and stored at 4 °C until use. All the glassware and other apparatus were sterilized by autoclave and used in dilution of soil samples. Samples were diluted up to 10^{-6} by serial dilution method. First step take 1 g of soil and 99 mL water in conical flask. Vortex sample and serially make further dilutions. Prepared samples 0.1 mL in quantity from last diluted step, which was used for further process.

Prepared starch casein agar media and sterilized by autoclave then added Nystatin (50 µg/mL) in petri-dishes under laminar air flow. Soil samples were added in 0.1 mL quantity and spread on the surface of media with help of sterile glass rod bent in structure. Then left at 28 °C for 1 week and observe growth of microbes on plates. By using streak plate method make pure colonies of actinomycetes using same media which colonies developed.

Characteristics of actinomycetes isolates

Colony characteristics: Based on appearance and morphological characters colonies of actinomycetes were selected and mycelium colour and physical structure were observed. Different types of appearance of colonies like smooth, lathery, dry *etc.* were studied and interpreted results of both set of samples [9].

Total 46 actinomycetes isolates were found by using 22 soil samples (11 soil samples from surrounding soil of medicinal plant area and 11 soil samples from not nearby medicinal plant area).

Microscopic examination: Cover slip cultures of isolates were prepared by using ISP agar media. Sterile cover slip used and inserted at 45° angle on the surface of media and after incubation plates were studied in respect to growth of colonies. Incubation duration was 7 days at 28 °C temperature. Study growth of microbes in cover slip by microscopic examination in respect to mycelium growth, sporulation and all morphological parameters [10].

Primary screening for antimicrobial activity: Based on colony characteristics all isolates were used for screening for antimicrobial activity. Cross streak method used for microbe's growth. There are different methods available for screening purpose but because of easy operation with fast results cross streak is widely used amongst other. Muller Hinton agar (MHA) media was prepared. Ingredients like meat, casein acid hydrolyzate, starch and agar used in composition of MHA media with pH 7.3 ± 0.1 at 25 °C. Autoclave used for sterilization of media at 121 °C for 30 min. After some time media solidify at room temperature, which were used for further inoculation process with actinomycetes cultures with single line streak in petri dishes centre after that incubated at 20 °C for 7 days. This allow well diffuse of microbes in media and good growth of them [11].

After observing a good growth on the petri plates, the test organisms were streaked at right angles to the streak of actinomycetes without touching central growth of actinomycetes and plates were incubated at 30 °C for 24 h. After incubation length of streak growth was measured. Different 8 pathogenic microbes used to check antimicrobial activity of isolates. Measure the inhibition area [12].

Secondary screening: Top 5 isolates were selected for secondary screening based on their antimicrobial activity. Prepared sterile MHA media and allow solidifying at room temperature. Inoculate different pathogens on plate and allow incubating at 20 °C for 7 days [13].

Make bore or well around 8 mm diameter in solid media with help of sterile stainless steel borer procedure done under aseptic chamber. Add 0.1 mL of isolate extract from selected isolates and kept for 37 °C for 7 to 9 days. Extract prepared from using 2.5 % microbial broth and centrifugation at 5000 rpm for 1 h. After duration of incubation observed growth of microbes and zone of inhibition surrounding well [14]. Disk diffusion method also used to check antimicrobial activity of selected actinomycete isolates. In this method disk were prepared using 1:1 volume of extracted isolate broth of samples. Zone of inhibition was measured surrounding disk of samples.

RESULTS AND DISCUSSION

Morphological characterization: Based on colony morphology 32 actinomycetes isolate observed from soil samples of 11 places which were not nearby medicinal plants and 36 actinomycetes isolate observed from soil samples collected surrounding 11 different medicinal plants.

Actinomycete isolates produced from soil nearby 11 different medicinal plants area showed arial mycelium white colour of colony in 20 (55.56 %) isolates, cream colour of colony in 5 (13.89 %) isolates, yellow colour of colony in 4 (11.11 %) of isolates, brown colour of colony in 3 (8.33 %) of isolates, gray colour of colony in 2 (5.56 %) isolates, pinkish colour of colony in 1 (2.78 %) of isolate and bluish colour of colony in 1 (2.78 %) of isolates.

Actinomycete isolates produced from soil nearby 11 different medicinal plants area showed vegetative mycelium yellow colour of colony in 16 (44.44 %) isolates, white colour of colony in 7 (19.44 %) isolates, cream colour of colony in 5 (13.89 %) of isolates, brown colour of colony in 4 (11.11 %) of isolates, gray colour of colony in 3 (8.33 %) isolates and redish colour of colony in 1 (2.78 %) of isolates.

Soil is rich source of different types of microorganism produced antimicrobial activity against many harmful pathogens [15,16]. Some study also shown that white colour of arial mycelium was dominant and yellow colour is in the case of vegetative mycelium colony [17]. Also more diversity of colony colour was observed in arial mycelium than vegetative mycelium colony [18]. Previous study shown that diversity to produce potent actinomycetes was depend on ecological regions [19].

Actinomycete isolates produced from soil not nearby medicinal plants area showed arial mycelium white colour of colony in 17 (53.13 %) isolates, cream colour of colony in 5 (15.63 %) isolates, yellow colour of colony in 4 (12.50 %) of isolates, brown colour of colony in 2 (6.25 %) of isolates, gray colour of colony in 2 (6.25 %) isolates, pinkish colour of colony in 1 (3.13 %) of isolate and bluish colour of colony in 1 (3.13 %) of isolates.

Actinomycete isolates produced from soil not nearby medicinal plants area showed vegetative mycelium yellow colour of colony in 15 (46.88 %) isolates, white colour of colony in 6 (18.75 %) isolates, cream colour of colony in 4 (12.50 %) of isolates, brown colour of colony in 4 (12.50 %) of isolates, gray colour of colony in 2 (6.25 %) isolates and redish colour of colony in 1 (3.13 %) of isolates.

Screening for antimicrobial activity: It was found that 36 actinomycetes isolates observed from soil surrounding medicinal plant area and all isolated used for primary screening against 8 test pathogens. It was found that against *Bacillus subtilis* 12 isolates not showed any activity, 5 isolates showed more than 20 mm ZOI, 11 isolates showed 15 to 20 mm ZOI, 3 isolates showed 10 to 15 mm ZOI and 5 isolates showed less

than 10 mm ZOI. Against *Staphylococcus aureus* 16 isolates showed not any activity, 6 isolates showed more than 20 mm ZOI, 9 isolates showed 15 to 20 mm ZOI, 2 isolates showed 10 to 15 mm ZOI and 3 isolates showed less than 10 mm ZOI. Against *Proteus vulgaris* 19 isolates showed not any activity, 3 isolates showed more than 20 mm ZOI, 5 isolates showed 15 to 20 mm ZOI, 3 isolates showed 10 to 15 mm ZOI and 6 isolates showed less than 10 mm ZOI. Against *Escherichia coli* 13 isolates showed not any activity, 3 isolates showed more than 20 mm ZOI, 10 isolates showed 15 to 20 mm ZOI, 1 isolates showed 10 to 15 mm ZOI and 9 isolates showed less than 10 mm ZOI.

Against *Klebsiella aerogenes* 14 isolates showed not any activity, 4 isolates showed more than 20 mm ZOI, 10 isolates showed 15 to 20 mm ZOI, 2 isolates showed 10 to 15 mm ZOI and 6 isolates showed less than 10 mm ZOI. Against *Pseudomonas aeruginosa* 21 isolates showed not any activity, 3 isolates showed more than 20 mm ZOI, 5 isolates showed 15 to 20 mm ZOI, 1 isolates showed 10 to 15 mm ZOI and 6 isolates showed less than 10 mm ZOI. Against *Salmonella typhi* 24 isolates showed not any activity, 0 isolates showed more than 20 mm ZOI, 5 isolates showed 15 to 20 mm ZOI, 1 isolates showed 10 to 15 mm ZOI and 6 isolates showed less than 10 mm ZOI. Against *Enterobacter aerogenes* 22 isolates showed not any activity, 0 isolates showed more than 20 mm ZOI, 4 isolates showed 15 to 20 mm ZOI, 2 isolates showed 10 to 15 mm ZOI and 8 isolates showed less than 10 mm ZOI (Table-1).

It was found that 36 actinomycetes isolates observed from soil not surrounding medicinal plant area and all isolated used for primary screening against 8 test pathogens. It was found that against *Bacillus subtilis*, 11 isolates not showed any activity, 4 isolates showed more than 20 mm ZOI, 10 isolates showed 15 to 20 mm ZOI, 2 isolates showed 10 to 15 mm ZOI and 5 isolates showed less than 10 mm ZOI. Against *Staphylococcus aureus* 14 isolates showed not any activity, 5 isolates showed more than 20 mm ZOI, 8 isolates showed 15 to 20 mm ZOI, 2 isolates showed 10 to 15 mm ZOI and 3 isolates showed less than 10 mm ZOI. Against *Proteus vulgaris* 17 isolates showed not any activity, 3 isolates showed more than 20 mm ZOI, 4 isolates showed 15 to 20 mm ZOI, 3 isolates showed 10 to 15 mm ZOI and 5 isolates showed less than 10 mm ZOI. Against *Escherichia coli* 11 isolates showed not any activity, 3 isolates showed more than 20 mm ZOI, 8 isolates showed 15 to 20 mm ZOI, 1 isolates showed 10 to 15 mm ZOI and 9 isolates showed less than 10 mm ZOI (Table-2).

TABLE-1
DISTRIBUTION OF ACTINOMYCETES ISOLATES PRODUCED FROM SOIL SURROUNDING
MEDICINAL PLANTS SHOWED ANTIMICROBIAL ACTIVITY AGAINST TEST PATHOGENS

Test pathogens	Not active	> 20 mm	15–20 mm	10–15 mm	< 10 mm	Total number of isolates
<i>Bacillus subtilis</i>	12	5	11	3	5	36
<i>Staphylococcus aureus</i>	16	6	9	2	3	36
<i>Proteus vulgaris</i>	19	3	5	3	6	36
<i>Escherichia coli</i>	13	3	10	1	9	36
<i>Klebsiella aerogenes</i>	14	4	10	2	6	36
<i>Pseudomonas aeruginosa</i>	21	3	5	1	6	36
<i>Salmonella typhi</i>	24	0	5	1	6	36
<i>Enterobacter aerogenes</i>	22	0	4	2	8	36

TABLE-2
DISTRIBUTION OF ACTINOMYCETES ISOLATES PRODUCED FROM SOIL NOT SURROUNDING
MEDICINAL PLANTS SHOWED ANTIMICROBIAL ACTIVITY AGAINST TEST PATHOGENS

Test pathogens	Not active	> 20 mm	15–20 mm	10–15 mm	< 10 mm	Total number of isolates
<i>Bacillus subtilis</i>	11	4	10	2	5	32
<i>Staphylococcus aureus</i>	14	5	8	2	3	32
<i>Proteus vulgaris</i>	17	3	4	3	5	32
<i>Escherichia coli</i>	11	3	8	1	9	32
<i>Klebsiella aerogenes</i>	12	4	9	2	5	32
<i>Pseudomonas aeruginosa</i>	19	3	4	1	5	32
<i>Salmonella typhi</i>	22	0	4	1	5	32
<i>Enterobacter aerogenes</i>	20	0	3	2	7	32

Against *Klebsiella aerogenes* 12 isolates showed not any activity, 4 isolates showed more than 20 mm ZOI, 9 isolates showed 15 to 20 mm ZOI, 2 isolates showed 10 to 15 mm ZOI and 5 isolates showed less than 10 mm ZOI. Against *Pseudomonas aeruginosa* 19 isolates showed not any activity, 3 isolates showed more than 20 mm ZOI, 4 isolates showed 15 to 20 mm ZOI, 1 isolates showed 10 to 15 mm ZOI and 5 isolates showed less than 10 mm ZOI. Against *Salmonella typhi* 22 isolates showed not any activity, 0 isolates showed more than 20 mm ZOI, 4 isolates showed 15 to 20 mm ZOI, 1 isolates showed 10 to 15 mm ZOI and 5 isolates showed less than 10 mm ZOI. Against *Enterobacter aerogenes* 20 isolates showed not any activity, 0 isolates showed more than 20 mm ZOI, 3 isolates showed 15 to 20 mm ZOI, 2 isolates showed 10 to 15 mm ZOI and 7 isolates showed less than 10 mm ZOI (Table-2).

Study shown that chances to produced actinomycetes isolates from agriculture soil was greater than non-agriculture soil isolates [20].

Some study states that there were marginal difference of actinomycetes isolates shown more than 15 mm ZOI from fertile and non fertile soil isolates [21-23]. Here we found that there were around 20 to 30 % more chances to show activity more than 15 mm of isolate produced from soil collected nearby medicinal plant area versus actinomycetes isolate produced from soil not nearby medicinal plant area.

Top 5 actinomycetes isolates selected from both sets for further process. Agar well diffusion method was used for secondary screening of most potent actinomycetes isolates.

The isolates (A)OS3 showed 20 mm ZOI against *Bacillus subtilis*, (B)TF2 showed 18 mm ZOI against *Bacillus subtilis*, (A)OS3 shows 21 mm ZOI against *Escherichia coli*, (B)RI1 showed 18 mm ZOI against *Escherichia coli* (Table-3).

Only five actinomycetes isolate shown antimicrobial activity against pathogens out 70 isolates produced from soil of Madhya Pradesh, India [2,12]. No actinomycetes isolated shown more than 20 mm ZOI against test pathogens used for antimicrobial activity out of 43 isolates of Saudi University garden area [6,24-26].

It was observed that isolate (A)OS1 showed 27.00 ± 1 ZOI against *Bacillus subtilis*, 22.00 ± 1 ZOI against *Staphylococcus aureus*, 24.67 ± 0.58 ZOI against *Proteus vulgaris*, 20.00 ± 1 ZOI against *Escherichia coli*, 26.00 ± 1 ZOI against *Klebsiella aerogenes*, 19.00 ± 1 ZOI against *Salmonella typhi* and 18.33 ± 0.58 ZOI against *Enterobacter aerogenes*. Isolate (A)OS3 showed 20.00 ± 1 ZOI against *Bacillus subtilis*, 20.33 ± 0.58 ZOI against *Staphylococcus aureus*, 21.33 ± 0.58 ZOI against *Proteus vulgaris*, 21.00 ± 1 ZOI against *Escherichia coli*, 18.67 ± 0.58 ZOI against *Klebsiella aerogenes*, 19.00 ± 1 ZOI against *Salmonella typhi* and 16.00 ± 1 ZOI against *Enterobacter aerogenes*. Isolate (A)AI1 showed 25.00 ± 1 ZOI against *Bacillus subtilis*, 19.33 ± 0.58 ZOI against *Staphylococcus aureus*, 18.67 ± 0.58 ZOI against *Proteus vulgaris*, 18.33 ± 0.58 ZOI against *Escherichia coli*, 20.00 ± 1 ZOI against *Klebsiella aerogenes*, 20.00 ± 1 ZOI against *Salmonella typhi* and 21.00 ± 1 ZOI against *Enterobacter aerogenes*. Isolate (A)TF1 showed 18.67 ± 0.58 ZOI against *Bacillus subtilis*, 20.33

TABLE-3
ZONE OF INHIBITION (ZOI) AGAINST TEST PATHOGENS OF BOTH SETS OF TOP 5 POTENT ISOLATES

Isolates code name	Microbial species							
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Proteus vulgaris</i>	<i>Escherichia coli</i>	<i>Klebsiella aerogenes</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella typhi</i>	<i>Enterobacter aerogenes</i>
Isolates produced from soil surrounding medicinal plant area								
(A)OS1	27.00 ± 1	22.00 ± 1	24.67 ± 0.58	20.00 ± 1	26.00 ± 1	0	19.00 ± 1	18.33 ± 0.58
(A)OS3	20.00 ± 1	20.33 ± 0.58	21.33 ± 0.58	21.00 ± 1	18.67 ± 0.58	0	19.00 ± 1	16.00 ± 1
(A)AI1	25.00 ± 1	19.33 ± 0.58	18.67 ± 0.58	18.33 ± 0.58	20.00 ± 1	0	20.00 ± 1	21.00 ± 1
(A)TF1	18.67 ± 0.58	20.33 ± 1.15	22.00 ± 2	20.00 ± 1	20.67 ± 0.58	22.33 ± 1.15	0	0
(A)RI1	20.67 ± 0.58	22.67 ± 0.58	23.00 ± 1	20.67 ± 1.15	21.33 ± 0.58	20.00 ± 1	0	0
Isolates produced from soil not surrounding medicinal plant area								
(B)3	19.67 ± 0.58	22.67 ± 1.53	0	20.00 ± 0.1	18.33 ± 0.58	19.67 ± 0.58	0	0
(B)7	19.33 ± 0.58	20.00 ± 1	0	18.00 ± 1	20.00 ± 1	19.67 ± 0.58	0	16.67 ± 0.58
(B)8	21.67 ± 0.58	17.33 ± 0.58	0	20.00 ± 1	20.00 ± 1	18.67 ± 0.58	0.00	15.33 ± 0.58
(B)16	21.00 ± 1	21.67 ± 0.58	0	20.00 ± 1	19.33 ± 0.58	17.00 ± 1	0	18.33 ± 0.58
(B)30	18.67 ± 0.58	19.33 ± 0.58	21.00 ± 1	22.00 ± 1	20.67 ± 0.58	19.33 ± 0.58	0	0

Values are mean $\pm$ SD of three replications.

± 1.15 ZOI against *Staphylococcus aureus*, 22.00 ± 2 ZOI against *Proteus vulgaris*, 20.00 ± 1 ZOI against *Escherichia coli*, 20.67 ± 0.58 ZOI against *Klebsiella aerogenes* and 22.33 ± 1.15 ZOI against *Pseudomonas aeruginosa*. Isolate (A)RII showed 20.67 ± 0.58 ZOI against *Bacillus subtilis*, 22.67 ± 0.58 ZOI against *Staphylococcus aureus*, 23.00 ± 1 ZOI against *Proteus vulgaris*, 20.67 ± 1.15 ZOI against *Escherichia coli*, 21.33 ± 0.58 ZOI against *Klebsiella aerogenes* and 20.00 ± 1 ZOI against *Pseudomonas aeruginosa*.

It was observed that isolate (B)1 showed 19.67 ± 0.58 ZOI against *Bacillus subtilis*, 22.67 ± 1.53 ZOI against *Staphylococcus aureus*, 20.00 ± 0.1 ZOI against *Escherichia coli*, 18.33 ± 0.58 ZOI against *Klebsiella aerogenes* and 19.67 ± 0.58 ZOI against *Pseudomonas aeruginosa*. Isolate (B)2 showed 19.33 ± 0.58 ZOI against *Bacillus subtilis*, 20.00 ± 1 ZOI against *Staphylococcus aureus*, 18.00 ± 1 ZOI against *Escherichia coli*, 20.00 ± 1 ZOI against *Klebsiella aerogenes*, 19.67 ± 0.58 ZOI against *Pseudomonas aeruginosa* and 16.67 ± 0.58 ZOI against *Enterobacter aerogenes*. Isolate (B)3 showed 21.67 ± 0.58 ZOI against *Bacillus subtilis*, 17.33 ± 0.58 ZOI against *Staphylococcus aureus*, 20.00 ± 1 ZOI against *Escherichia coli*, 20.00 ± 1 ZOI against *Klebsiella aerogenes*, 18.67 ± 0.58 ZOI against *Pseudomonas aeruginosa* and 15.33 ± 0.58 ZOI against *Enterobacter aerogenes*. Isolate (B)4 showed 21.00 ± 1 ZOI against *Bacillus subtilis*, 21.67 ± 0.58 ZOI against *Staphylococcus aureus*, 20.00 ± 1 ZOI against *Escherichia coli*, 19.33 ± 0.58 ZOI against *Klebsiella aerogenes*, 17.00 ± 1 ZOI against *Pseudomonas aeruginosa* and 18.33 ± 0.58 ZOI against *Enterobacter aerogenes*. Isolate (B)5 showed 18.67 ± 0.58 ZOI against *Bacillus subtilis*, 19.33 ± 0.58 ZOI against *Staphylococcus aureus*, 21.00 ± 1 ZOI against *Proteus vulgaris*, 22.00 ± 1 ZOI against *Escherichia coli*, 20.67 ± 0.58 ZOI against *Klebsiella aerogenes* and 19.33 ± 0.58 ZOI against *Pseudomonas aeruginosa*.

It was found that actinomycetes isolates produced from soil surrounding medicinal plant area total 36 isolates obtained amongst them 26 (72.22 %) isolates had antimicrobial activity against test pathogens and 10 (27.78 %) isolates not have any activity. Thirty two actinomycetes isolates produced from soil not nearby medicinal plant area amongst them 17 (53.12 %) showed antimicrobial activity against test pathogens and 15 (46.88 %) not showed any activity. Some study shown that *Rosa indica* plant soil area is rich source of actinomycetes isolates, which have potent antimicrobial activity. In our study also 3 isolates out of 10 most potent isolates were from soil nearby *Rosa indica* [3,6,27].

Conclusion

This research concluded that more number of actinomycetes isolates produced from soil surrounding medicinal plant area than soil not nearby medicinal plants of Junagadh area of Gujarat state. Actinomycetes isolates shown greater than 20 mm ZOI from soil surrounding medicinal plant as compare isolates produced from soil not nearby medicinal plant. Only 28 % of actinomycetes isolates produced from soil surrounding medicinal plant area had not shown any activity against pathogens while isolates produced from soil not nearby medicinal plant shown 47 % of isolates had not any activity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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