WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 3, 1064-1072. Research Article ISSN 2277– 7105

STUDY OF ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANT RHIZOSPHERE SOIL STREPTOMYCES ON SOME HUMAN SKIN PATHOGENS

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Article Received on
07 Dec. 2017,

Revised on 07 Jan. 2018,
Accepted on 27 Jan. 2018

DOI: 10.20959/wjpr20183-10896

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ABSTRACT

Actinomycetes are Gram-positive rod shaped, filamentous organisms which have ability to grow slowly as branching filaments. Most commonly soil isolated actinomycetes are of genus *Streptomycetes*. The aim of present study is to search antimicrobial activity produced by *Streptomyces* against some human skin pathogens. Total five rhizosphere soil samples of medicinal plants viz; *Aloe barbadense* (Aloevera), *Emblica officinalis* (Avala), *Zingiber officinale* (Ginger), *Eucalyptus camaldulensis* (Nilgiri) and *Santalum album* (Sandal) were collected from local area at Barshi, Dist. Solapur. M.S. India. Total thirty-two isolates were obtained and studied as actinomycetes on the basis of morphologically, culturally and biochemically characters. Out of these, nineteen isolates were identified from genus *Streptomyces* by MICRO-IS Software and also few by16srRNA analysis. The obtained isolates were tested for their antimicrobial activity by agar overlay technique against *Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa*. The zone of inhibition around the growth of isolates were measured and recorded as a sensitive, while no zone of inhibiton around the isolate were consider as resistant to that test organism. Out of these isolates, five *Streptomyces* isolates were showed maximum antimicrobial activity against all tested skin pathogens *Streptomyces* enissocaesilis was showed antimicrobial activity against all pathogens except *Pseudomonas aeruginosa*. *Streptomyces flavoviridis* was showed antimicrobial activity against all tested human skin pathogens except *Pseudomonas aeruginosa* and *Escherichia coli.*

KEYWORDS: *Streptomyces*, skin pathogens, agar overlay technique.

INTRODUCTION

The soil is the largest reservoir of medicinally important microorganisms. The actinobacteria are Gram- positive, aerobic and spore forming. The actinomycetes found in various habitat in nature (George *et.al*, 2012). Most actinomycetes in soil are belong to the genus *Streptomyces* (Good fellow and Simpson, 1987). *Streptomyces* are belonging to the family Streptomycetaceae. Within this family, 500 species are described. The characteristics "Geosmin" (earthy odour) of soil is caused by metabolite production of *Streptomyces*. The *Streptomyces* are most important and widely used in industry because the ability to produce many chemical compounds like antibiotics, enzymes and anti-tumor agents (Berdy, 1995).

Micro-organisms found in large numbers in and on humans and cause various infections and diseases. Skin is common sites of bacterial and fungal infections. It is the body's first line of defense mechanism against pathogens. Normally, the skin infection may involve the superficial layers (epidermis) only but may involve the deeper dermis (sweat glands, oil glands, lymphatic and hair follicles) and mostly by an injury or abrasion. *Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* are known skin commensals but exhibit pathogenic potential under certain conditions. These pathogens live on human skin without causing disease. However, when person's skin is broken or cut, they can enter the wound and cause an infection. *Escherichia coli* is common resident of intestinal tract, can be acquired due to poor personal hygiene. *Staphylococcus aureus* is found particularly among openings such as nose, mouth, and genitals. When skin is cut, these bacteria can enter the wound and some strain of *Staphylococcus* produce a toxin that causes illness. *Klebsiella pneumoniae* is a causative agent of nosocomial infection and found in the normal flora of the mouth, skin and intestine. *Klebsiella pneumoniae* can progress into severe bacterial infection like wound infections. *Pseudomonas aeruginosa* is found on skin especially in the axillary and anogenital regions. It is opportunistic pathogen and causes the disease in condition of immunocompromised. *Proteus vulgaris* is inhabits the intestinal tracts of human and animals and cause wound infection. In literature, some *Proteus* species are found in hospitals and they cause clinical infections. Although most skin infections are not serious, they can be very dangerous if left untreated.

Streptomyces are numerous in plant rhizosphere (Suzuki *et. al*, 2000). *Streptomyces* associated in rhizosphere soils of medicinal plants may be attractive source of novel bioactive compounds (Thangapandian *et.al*, 2007). Medicinal plants have play most useful role in traditional medicine. The plant derived drugs also known as "green medicine" is safe than costly synthetic drugs. Any part of the medicinal plants may contain active components like antimicrobial substance. These can be derived from stems, barks, leaves, flowers and fruits of plants (Gordon MC and David JN, 2001). *Aloe barbadense* (Aloe Vera) is a medicinal plant and it belongs to family Liliaceae and Aloeaceae which has numerous species. Aloe Vera is one of the most widely used herbal preparation for the treatment of various skin conditions.It contains treasures of nutritional and antipathogenic compounds. These nutrients are helpful as heal, moisturize and regenerate the skin (Davis *et., al*, 1989 a, Zawahry *et., al*; 1973). It has antibacterial, antifungal and antiviral properties.

Emblica officinalis (Avala) is a well-known medicinal plant. It belongs to family Euphorbeaceae. The species native to India, tropical and subtropical regions. The active principles of Avala is an excellent therapeutic formulation for infected wounds (Kumar, M.S.*et., al,* 2008). *Zingiber officinale* (Ginger) is from family Zingiberaceae. It is originate in China and spread to India, South East Asia and West Africa. The antimicrobial activity of *Zingiber officinale* may be increased due to antimicrobial substances such as zingiberol, zingiberine and biosabolene (Michael derrida, 1999; Melvin *et., al*, 2009). *Eucalyptus camaldulensis* (Nilgiri) is a medicinal plant from family Myrtaceae. The species of this family are native of Australia and widely cultivated throughout the tropics (Asia, Central America as well as Africa). Plants are used to treat enteric infection, boils, skin and wound infections (Bala, 2006). *Santalum album* (Sandal) is from family Sanctaceae. This medicinal plant is native to India, Indonesia and Malay Archipelago. *Santalum album* is usually used to treat skin itching, inflammation (Chopra *et., al*, 1982).

Due to the increasing multidrug resistance of pathogens, there is constant need to discover alternative compounds for treatment of infectious diseases (Gull *et. al*, 2012). There are few reports on antimicrobial substances from rhizosphere soil of medicinal plant. Hence the locally available some medicinal plants are selected and the antimicrobial activity tested against some skin pathogens.

MATERIALS AND METHODS

Collection of soil

Rhizosphere soils from *Aloe barbadense* (Aloevera), *Emblica officinalis* (Avala), *Zingiber officinale* (Ginger), *Eucalyptus camaldulensis* (Nilgiri) and *Santalum album* (Sandal) plants were available local area at Barshi, Dist. Solapur, M.S. India. The soil samples were collected at depth of 10-20 cm from selected medicinal plants. Soil samples were collected in sterile polythene bags, labelled, brought to the laboratory and used in present study.

Test organisms

Standard culture of test organisms (24 hrs. old culture) such as *Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* were obtained from local hospital source, confirmed and then used.

Isolation of actinomycetes

For this study, five soil samples were collected and transported to sterile polythene bag and air dried at room temperature. Collected soil samples were suspended in sterile distilled water (1gm in 100 ml) and 0.1 ml of serially dilution transferd to glycerol asparagine agar (L-asparagine -0.1g, K_2HPO_4 -0.1g, glycerol-1gm, trace salt solution-0.1ml, agar-2.5, distilled water-100ml, pH-7.4). After incubation distinct leathery colonies were selected and subcultured on glycerol asparagine agar slants. Pure culture were maintained on glycerol asparagine agar slants and stored at 4^0C for further study.

Identification of actinomycetes

The isolates were selected on the basis of cultural characteristics like leathery or powdery colonies. The selected isolates were identified as *Streptomyces* on the basis of morphological characters (cell wall, aerial mycelium, substrate mycelium and sporulation) by coverslip culture technique and also by biochemical characeristics. *Streptomyces* were identified to genus and species level by MICRO-IS Software and few also by16srRNA analysis.

Antimicrobial activity Test

For this study, Agar overlay technique (Bergey, 1983) were used for antimicrobial test. This technique have ability to produce any antibiotic using test organisms. The spot of isolate were inoculated on glycerol asparagine agar. After incubation of ambient temperature for 4-7 days, the growth of isolates on medium were killed by inverting the plates over petri dish containing 1.5 ml chloroform for 40 min. After removal of excess chloroform vapors, the

surface was overlaid with 5 ml of 0.7% w/v nutrient agar (Peptone-1g, NaCl-0.5g, Yeast extract-0.3, agar -0.7 g, distilled water-100ml pH- 7) and incubated with 0.2ml of suspension containing bacterial test organism. After incubation, the zone diameter of inhibition in mm around the isolate were measured as sensitive or resistance pattern.

RESULTS AND DISCUSSION

Total thirty- two isolates were isolated from rhizosphere soil samples of medicinal plants viz; *Aloe barbadense* (Aloevera), *Emblica officinalis* (Avala), *Zingiber officinale* (Ginger), *Eucalyptus camaldulensis* (Nilgiri) and *Santalum album* (Sandal) rhizosphere soil samples. *Streptomyces* are abundant in the plant rhizosphere. Glycerol asparagine agar medium provides glycerol and asparagine as carbon and nitrogen source respectively. The powdery growth of colony were selected for the further study.

Table 1: Antimicrobial activity of selected *Streptomyces* against pathogens by Agar overlay technique.

Sr. No	Streptomyces	Zone diameter of inhibition against pathogens in mm				
		SA	PV	KP	EC	PA
1.	*Streptomyces enissocaesilis*	50 (10)*	28 (10)*	22 (10)*	15(10)*	16(10)*
2.	*Streptomyces xanthochromogenes*	40 (10)*	26 (10)*	R	R	R
3.	*Streptomyces antibioticus*	30 (10)*	20 (10)*	19(10)*	18 (10*)	R
4.	*Streptomyces flavoviridis*	45 (10)*	35(10)*	20(10)*	R	R
5.	*Streptomyces aureofaciens*	46(10)*	30(10)*	25(10)*	20(10)*	12(10)*

SA = *Staphylococcus aureus*, PV = *Proteus vulgaris*, KP = *Klebsiella pneumoniae*,

EC = *Escherichia coli*, PA = *Pseudomonas aeruginosa*

*= Zone of growth of isolates on media.

R = Resistance (No zone around the isolate)

Out of thirty – two isolates, nineteen isolates were identified as *Streptomyces* and remaining from genus *Streptoverticillium, Nocardia, Micromonospora and Micropolyspora*. Out of nineteen *Streptomyces* species, five *Streptomyces* species were showed maximum zone of inhibition. Table 1 represents the *Streptomyces* and their antimicrobial activity against pathogens. Total five *Streptomyces* showed antimicrobial activity viz; *Streptomyces enissocaesilis, Streptomyces xanthochromogenes, Streptomyces antibioticus, Streptomyces flavoviridis* and *Streptomyces aureofaciens*. In antimicrobial activity test, *Streptomyces enissocaesilis* and *Streptomyces aureofaciens* showed antimicrobial activity against all tested pathogens. *Streptomyces xanthochromogenes* showed antimicrobial activity against

Staphylococcus aureus and *Proteus vulgaris*. *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* found resistant. *Streptomyces antibioticus* showed antimicrobial activity against all tested pathogens except *Pseudomonas aeruginosa*. *Streptomyces flavoviridis* showed antimicrobial activity against *Staphylococcus* aureus, *Proteus vulagaris* and *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* found resistant. Antimicrobial activity of all *Streptomyces* isolates by agar overlay technique were shown in Table.1.

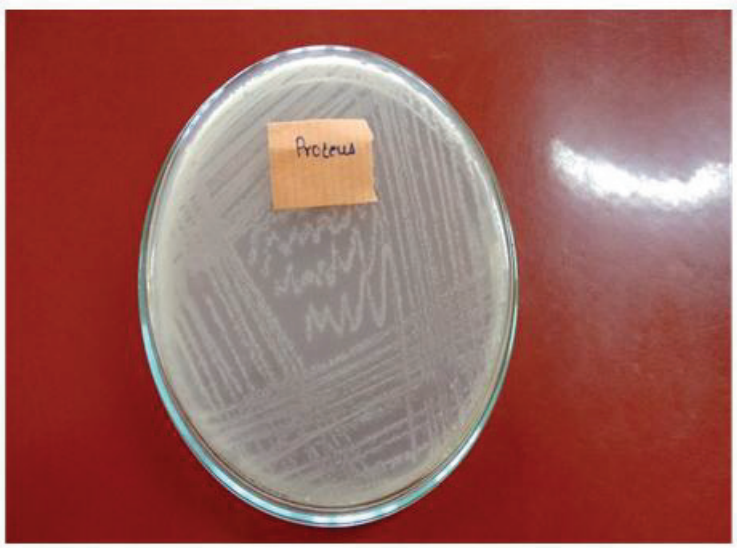

Plate 1: Isolation of *Proteus vulgaris*.

The standard culture of test organisms such as *Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* were used as test organisms. These pathogens normally found on and in human body and doesn't harm but at certain condition they pass through skin by an injury and cause infection. Plate -1 shows the isolation of *Proteus vulgaris* as a test organism on medium.

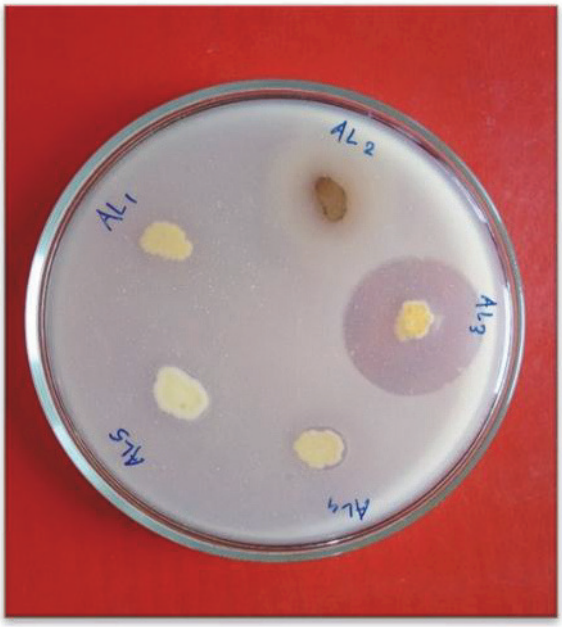

Plate – 2. **Plate – 3.**

Plate 2 and 3: Antimicrobial activity of *Streptomyces enissocaesilis* on *Staphylococcus aureus* and *Proteus vulgaris* respectively.

The antimicrobial substance produced by *Streptomyces enissocaesilis* was showed the antimicrobial activity against all pathogens viz; *Staphylococcus aureus*, *Proteus vulgaris, Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa.*

The formation of inhibition zone around the pathogenic strain is due to the production of secondary metabolite by *Streptomyces* species (Demain, 1983, Sanglier *et., al*, 1993).

There are twenty thousand known secondary metabolites which drawn from medicinal plants and used to fight infections and diseases.In fact, 70-80% of the commercially available secondary metabolites have been isolated and characterized by several species of actinomycetes (Khanna *et., al,* 2011). The most antibiotics developed for human pharmaceutical uses are derived from *Streptomyces* species (Good fellow *et., al*, 1987). The bacterial resistance to antibiotic is a major problem today. There is constant need to search of new antimicrobial compounds with novel mechanisms of action for multidrug resistance pathogens and infectious diseases. It is possible by using various medicinal plants and their parts for medicinally valuable compounds.

There are many reports in the literature regarding the study of antimicrobial activity and actinomycetes as source of antimicrobial substances viz; Kamble and Kulkarni (2014),

Khanna *et., al.* (2011), Melvin *et.al* (2009), Sanglier *et., al* (1993). So far no one had reported medicinal rhizospheric actinomycetes as source of antimicrobial substances from Barshi region. Hence our reports stand as maiden report. According to this study, medicinal plants would be the best source to obtain a variety of antimicrobial substances.

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