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# A PRELIMINARY STUDY ON THE SECONDARY METABOLITES FROM SOIL BACTERIAL SPECIES

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## ABSTRACT
Secondary metabolites are low molecular mass/weight products of secondary metabolism, not essential for the growth of producing microorganism but very important for the human health. The objective of this work is the isolation and identification of microbes from different polluted soil sample. Four organisms were selected to determine the ability to produce secondary metabolites. The isolated bacteria were identified as *Bacillus* sp1, *Bacillus* sp2, *Bacillus*3, *Pseudomonas* sp. From the antibiotic sensitivity assay tests, most of the metabolites are able to inhibit the growth of the other bacteria. Metabolites produced by *Bacillus* sp has better antibacterial activity than the others both in 7$^{th}$ day and 14$^{th}$ day incubation. These secondary products may be used as a novel antibiotics in future.

**KEYWORDS:-** Secondary Metabolite, Solvent Extraction, Antibacterial Activity, TLC.

## INTRODUCTION
The secondary metabolites are the organic compounds and are used to defend against predators, parasites, diseases and for interspecies competition, to facilitate the reproductive processes (coloring agents, attractive smells, etc). Secondary metabolites are not directly involved in the growth, development and reproduction of organisms.[1] Secondary metabolites are produced by some of the organisms to inhibit the growth of other organism competing for same ecological niche. These compounds are produced after active growth of an organism and are broad spectrum of structurally diverse secondary metabolites.[2] The secondary metabolites having various biological activities like, antimicrobial agents, inhibitors of enzymes and antitumor, immunosuppressive and antiparasitic agents.[3] Secondary metabolites are generally produced following active growth, and many have an unusual chemical structure and some are toxic to humans and other animals.[4]

## MATERIALS AND METHODS
### SAMPLE COLLECTION AND ISOLATION OF MICROORGANISMS
Polluted soil samples were collected from different places nearby our college and the isolation of organisms was done by using serial dilution method.

### PRODUCTION OF SECONDARY METABOLITES FROM THE BACTERIA
Prepare LB broth and selected colonies of *Bacillus* sp1, *Bacillus* sp2, *Bacillus* sp3, *Pseudomonas* sp, were inoculated in to separate flasks and were kept in the shaking incubator for 14 days at 180rpm. After 7$^{th}$ days and 14$^{th}$ days secondary metabolites were extracted.

### EXTRACTION OF SECONDARY METABOLITES BY SOLVENT EXTRACTION METHOD
After the production of secondary metabolites the each broth was aseptically transferred to separated sterile centrifuge tubes and centrifuged at 10,000 rpm for 10 minutes. The supernatant was transferred to the separating funnel and mixed with ethyl acetate .Then the mixture in the separating funnel was shaking continuously for 15 minutes. After 15 minutes keep the separated funnel undisturbed for 24 hours, three layers were formed and the middle layer was removed in the suspension was allowed to dry in the air. Dried sample contains the secondary metabolites and the weight was calculated.

### THIN LAYER CHROMATOGRAPHY
The silica gel plates were prepared and the samples were spotted gently in small amounts of using micropipette or capillary tube or syringe. 200 ml of solvent was prepared in a tank and spotted sample was placed vertically. When the solvent moved to the top, the plate is removed and allowed to dry at room temperature. The Rf value was measured by distance travelled by the sample and the

distance travelled by the solvents from the start point. The separation was measured in terms of a unit called using the formula,

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

**ANTIBACTERIAL ACTIVITY BY WELL DIFFUSION METHOD**

The test organisms *E. coli. Staphylcoccus aureus, Klebsiella* sp and *Pseudomonas* sp.were uniformly spread on Muller hinton agar medium. The wells were inoculated with respective volume of samples such as 25 µl,50 µl, 75 µl,100 µl and the plates were incubated at 37$^{o}$C for 24hrs. Examined the plates and measured the diameter of the inhibition zones.

**RESULTS**

**SAMPLE COLLECTION AND ISOLATION OF MICROORGANISMS**

4 different bacterial species which were repeated in all dilution plates were selected for the present study and identified as *Bacillus* sp1, *Bacillus* sp2, *Bacillus* sp3, and *Pseudomonas* sp.

**PRODUCTION OF SECONDARY METABOLITES FROM THE BACTERIA**

The secondary metabolites were produced from the study organisms such as *Bacillus* sp1, *Bacillus* sp2, *Bacillus* sp3 and *Pseudomonas* sp (Table.1), After 7$^{th}$ days and 14$^{th}$ days, secondary metabolites were extracted and were varies in quantity in 7$^{th}$ and 14$^{th}$ days of incubation time. Some isolates of *Streptomyces* species able to produce more than 180 different secondary metabolites.[5] The dry weight of secondary metabolites produced by all the organisms increased with increase in incubation time. The quantity of metabolites were higher in 14$^{th}$ days of incubation than 7$^{th}$ day. The maximum secondary metabolite was produced by *Bacillus* sp 2, 0. 8.1g/L at14$^{th}$ days of incubation. Lesser metabolite was produced at14$^{th}$ days of incubation by *Bacillus* sp 3, 1.0 g/L.

**THIN LAYER CHROMATOGRAPHY**

Thin Layer Chromatogram of secondary metabolites from *Bacillus* sp1, *Bacillus* sp2, *Bacillus* sp3, *Pseudomonas* sp showed the different Rf values in 7$^{th}$ day 14$^{th}$ day (Table.2). The separation of metabolite compounds of all organisms were clearly showed a spots in plates with the solvent Butanol. Hexane was failed to produce chromatogram on plate. Related study on the secondary metabolites of *Streptomycetes* sp and found that, metabolites could be either antibiotics or other secondary metabolites based on TLC results of different spots in 10 different isolates.[6]

**ANTIBACTERIAL ACTIVITY BY WELL DIFFUSION METHOD**

The antibacterial activity of the secondary metabolites produced by *Bacillus* sp1 (Table.3)., *Bacillus* sp2 (Table.4)., *Bacillus* sp3 (Table.5), *Pseudomonas* sp (Table. 6), were showed various bactericidal activities in7$^{th}$ and 14$^{th}$ day in all the volumes of the samples . The highest zone of inhibition was showed by *Bacillus* sp3 in 7$^{th}$ day tested on *Staphylcoccus* sp with zone of inhibition 41mm in100 µl and lowest inhibition showed by *Bacillus* sp-3 tested on *E. coli* with zone of 2 mm in 25 µl in 7$^{th}$ day. *Proteobacterium* sp produces secondary metabolite with antibacterial activity against both gram positive and gram negative organisms including methicilllin resistant *Staphylococcus aureus*.[7] 395 pure strains of East China Sea microorganism were isolated and 100 strains possess different biological activities and besides, also isolated macrolactin A and macrolactin B from *Bacillus subtilis*. *Sulfitobacter* sp, *Halomonas sp*, *Bacillus sp*, *Pseudoalteromonas sp* and *Idiomarina sp* and found that, biological activities, like antibacterial, cytotoxicity and antioxidant activities due to their secondary metabolites.[8] In 2007, a new macrolactin which was isolated from a marine *Bacillus subtilis*.[9]

**Table- 1- DETERMINATION OF DRY WEIGHT**

| S.NO | ORGANISM | DRY WEIGHT (gm) | |
|---|---|---|---|
| | | 7$^{th}$ day | 14$^{th}$ day |
| 1 | ***Bacillus* sp- 1** | **0.6** | **1.1** |
| 2 | ***Bacillus* sp- 2** | **0.62** | **8.1** |
| 3 | ***Bacillus* sp-3** | **0.4** | **1.0** |
| 4 | ***Pseudomonas* sp** | **0.3** | **1.3** |

**Table- 2- DETERMINATION OF RF VALUE**

| S.NO | ORGANISM | Rf VALUE | |
|---|---|---|---|
| | | 7$^{th}$ day | 14 $^{th}$ day |
| 1 | ***Bacillus* sp- 1** | **0.80** | **0.64** |
| 2 | ***Bacillus* sp- 2** | **0.85** | **0.73** |
| 3 | ***Bacillus* sp-3** | **0.69** | **0.67** |
| 4 | ***Pseudomonas* sp** | **0.59** | **0.61** |

**Table- 3- ANIBACTERIAL ACTIVITY OF THE SECONDARY METABOLITE - *BACILLUS* SP 1**

| S.NO | ORGANISM | TESTED AGAINST | ZONE OF INHIBITION ( mm) VOLUME OF SAMPLE | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 25µl | | 50 µl | | 75 µl | | 100 µl | |
| | | | 7th | 14th | 7th | 14th | 7th | 14th | 7th | 14th |
| 1 | *Bacillus sp*-1 | *E.coli.* | 4 | 7 | 7 | 19 | 9 | 11 | 10 | 6 |
| 2 | | *Staphylcoccus* sp | 4 | - | 6 | - | 10 | | 14 | - |
| 3 | | *Klebsiella* sp | 3 | | 4 | | 9 | | 15 | |
| 4 | | *Pseudomonas* sp | - | - | - | - | - | - | - | - |

- Zone Not Produced.

**Table- 4- ANTIBACTERIAL ACTIVITY OF THE SECONDARY METABOLITE - *BACILLUS* SP- 2**

| S.NO | ORGANISM | TESTED AGAINST | ZONE OF INHIBITION ( mm) VOLUME OF SAMPLE | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 25 µl | | 50 µl | | 75 µl | | 100 µl | |
| | | | 7th | 14th | 7th | 14th | 7th | 14th | 7th | 14th |
| 1 | *Bacillus sp*-2 | *E.coli* | 4 | - | 14 | - | 24 | - | 27 | - |
| 2 | | *Staphylcoccus* sp | 6 | 14 | 9 | 26 | 20 | 34 | 24 | 36 |
| 3 | | *Klebsiella* sp | 5 | - | 6 | - | 18 | - | 21 | - |
| 4 | | *Pseudomonas* sp | - | - | - | - | - | - | - | - |

**Table- 5- ANTIBACTERIAL ACTIVITY OF THE SECONDARY METABOLITE - BACILLUS SP- 3**

| S.NO | ORGANISM | TESTED AGAINST | ZONE OF INHIBITION ( mm) VOLUME OF SAMPLE | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 25 µl | | 50 µl | | 75 µl | | 100 µl | |
| | | | 7th | 14th | 7th | 14th | 7th | 14th | 7th | 14th |
| 1 | *Bacillus* sp 3 | *E.coli* | 2 | 21 | 6 | 23 | 9 | 25 | 20 | 27 |
| 2 | | *Staphylcoccus* sp | 5 | 12 | 25 | 17 | 34 | 21 | 41 | 19 |
| 3 | | *Klebsiella* sp | 10 | 20 | 21 | 21 | 25 | 24 | 29 | 25 |
| 4 | | *Pseudomonas* sp | 10 | 16 | 16 | 24 | 17 | 29 | 18 | 35 |

***BACILLUS* SP- 3.**

**Table- 6 - ANTIBACTERIAL ACTIVITY OF THE SECONDARY METABOLITE - *PSEUDOMONAS* SP**

| S.NO | ORGANISM | TESTED AGAINST | ZONE OF INHIBITION ( mm) VOLUME OF SAMPLE | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 25µl | | 50 µl | | 75 µl | | 100 µl | |
| | | | 7th | 14th | 7th | 14th | 7th | 14th | 7th | 14th |
| 1 | *Pseudomonas* sp. | *E.coli* . | | | | 14 | | 19 | | 21 |
| 2 | | *Staphylcoccus* sp | | 10 | | 20 | 30 | 32 | 15 | 35 |
| 3 | | *Klebsiella* sp. | 10 | 5 | 12 | 14 | 14 | 14 | 10 | 19 |
| 4 | | *Pseudomonas* sp. | 12 | 16 | 15 | 18 | 20 | 24 | 25 | 31 |

## CONCLUSION

Bacteria has high production of secondary metabolites with novel structures. In the current study secondary metabolites produced by using 4 bacterial species with higher antibacterial activity against some bacterial species. Further studies are ongoing to purify the antibacterial products. In future, these organisms may used as an antibacterial agents in the field of medicine.

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