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Research Article

Antimicrobial Screening of Wild *Solanum* Species against Human Respiratory Tract Infecting Biota by Bioautography Method

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ABSTRACT

In current world Natural products from plants are of keen interest for the discovery of antimicrobial compounds in the field of medicine. The present study was aimed to evaluate the antimicrobial activities against selected seven human respiratory tract infecting biota in three wild *Solanum* species namely *Solanum indicum, Solanum trilobatum* and *Solanum xanthocarpum* which are widely used in folk and traditional medicine to cure fever, whooping cough, bronchitis and common cold. The organic solvents such as Hexane, Acetone and Ethanol extract of leaves and fruits were used to find out the virulent activity of plant extracts on human pathogens. The standard disc diffusion assay was performed and the best five solvent extracts had chosen to perform TLC. Out of five solvents extracts three was chosen for direct bioautography study through agar overlay assay. All the extracts of the plants were tested against gram- positive and gram-negative microorganism to know there antimicrobial properties. Of these, leaf and fruit extracts of *Solanum indicum*, leaf of *Solanum trilobatum* exhibited maximum inhibition of the microbial growth than *Solanum xanthocarpum* extract.

Keywords: Antimicrobial, *Solanum sp.*, Plant extract, disc diffusion assay, bioautography, agar overlay assay.

INTRODUCTION

Medicinal plants have been used for several years in daily life to treat various diseases all over the world. Impressive numbers of modern drugs have been isolated from natural source and many are based on their use in traditional medicine. About 80% of individuals from developed countries using traditional medicine, which has been seen as a valuable source of medicine with proven potential of treating infectious diseases[1]. Compared to the synthetic drug agents plant compounds have lesser side effects. According to world health organization, medicinal plant would be the source to obtain a variety of drugs. Therefore, importance should be given to investigate their properties, safety and efficiency[2] and to provide the impetus to the search for novel substances from various sources of medicinal plants[3]. Several medicinal plants have been screened for their antimicrobial and antiviral properties[4]. Since plants produced a diverse range of bioactive molecules they will play a dominant role in the maintenance of human health[5]. The number of multi drug resistant microbial strains and their susceptibility to antibiotic are continuously increasing. This increase has been attributed to indiscriminate use of broad spectrum of using antibiotics and immune suppressive agents. There is an urgent need for the development of indigenous alternative antimicrobial molecules for the effective treatments of some serious diseases in the light of growing cases of microbial resistance to the time honoured antibiotics[6]. Almost as soon as antibacterial drug were developed, bacteria responded by manifesting various forms of resistance[7]. In this the wild plants which are used in traditional medicine such as *Solanum indicum, Solanum trilobatum, Solanum xanthocarpum* was further studied in order to insights the antimicrobial properties of the metabolites of the plants. Which help in identification of potential compounds and development of drug against Respiratory tract infecting biota.

MATERIALS AND METHODS

Plant Material Collection

The plant samples like leaves and fruits of *Solanum xanthocarpum, Solanum trilobatum* and *Solanum indicum* were collected from Eastern Ghats of Krishnagiri District, Tamil Nadu. The plant was identified and confirmed by Dr. G. Jeya Jothi, Assistant Professor, Department of Plant Biology and Biotechnology, Loyola College, Chennai.

Extraction Method

Leaves and fruits of *Solanum xanthocarpum, Solanum trilobatum* and *Solanum indicum* were shade dried and subsequently powdered and packed in air tight containers. Powdered plant material (20g) was soaked in 100% solvents at room temperature for three days on a rotary shaker (120rpm). Sequential extraction was carried out starting from low polar solvent to highly polar solvents. All extract were filtered through Buchner funnel with Whatman's filter Paper No.1 and the extracts were dried to remove the solvent and stored in brown bottles.

Culture of Microorganisms

The human respiratory tract infecting microorganisms were employed in the screening. Gram-positive and Gram-negative microorganisms used in this study were collected from Department of Plant Biology and Biotechnology

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Table 1: In vitro antimicrobial activity of crude extract from the leaves of *Solanum trilobatum* (Linn) Jack, Solanum indicum(Linn)Jack and *Solanum xanthocarpum* (Schard& H. wendl) Jack by disc diffusion method.

S.No	Microorganisms	*S. trilobatum*			*S. indicum*			*S. xanthocarpum*			CN
		HX	AC	EA	HX	AC	EA	HX	AC	EA	
1	*Streptococcus pneumonia*	NZ	10	9	10	8	NZ	10	9	9	NZ
2	*Streptococcus pyogenes*	NZ	10	NZ	NZ	18	NZ	14	NZ	NZ	10
3	*Klebsiella pneumonia*	NZ	NZ	NZ	8	8	NZ	NZ	NZ	9	10
4	*E. coli*	9	NZ	NZ	NZ	NZ	NZ	NZ	10	10	10
5	*Pseudomonas aeruginosa*	13	11	9	NZ	8	NZ	10	NZ	10	11
6	*Salmonella typhi*	NZ	8	NZ	11	8	14	16	NZ	12	35
7	*Salmonella paratyphi*	NZ	8	NZ	10	10	9	NZ	NZ	6	16

NZ: No zone, HX: Hexane, AC: Acetone, EA: Ethanol, CN: Control Disc content: Crude extracts3.0 mg/disc; Control: Erythromycin 15mg/disc

Table 2: In vitro antimicrobial activity of crude extract from the fruits of *Solanum trilobatum* (Linn) Jack, Solanum indium(Linn)Jack and *Solanum xanthocarpum* (Schard& H. wendl) Jack by disc diffusion method.

S.No	Microorganisms	*S. trilobatum*			*S. indicum*			*S. xanthocarpum*			CN
		HX	AC	EA	HX	AC	EA	HX	AC	EA	
1	*Streptococcus pneumonia*	NZ	NZ	NZ	NZ	NZ	8	NZ	9	9	NZ
2	*Streptococcus pyogenes*	10	10	14	10	16	20	10	15	11	10
3	*Klebsiella pneumonia*	NZ	NZ	NZ	NZ	10	NZ	NZ	NZ	NZ	10
4	*E. coli*	10	10	13	NZ	NZ	NZ	8	NZ	NZ	10
5	*Pseudomonas aeruginosa*	8	10	10	8	10	12	9	NZ	8	11
6	*Salmonella typhi*	10	NZ	10	10	8	12	8	NZ	10	35
7	*Salmonella paratyphi*	NZ	NZ	NZ	10	NZ	NZ	10	NZ	NZ	16

NZ: No zone, HX: Hexane, AC: Acetone, EA: Ethanol, CN: Control Disc content: Crude extracts3.0 mg/disc; Control: Erythromycin 15mg/disc

Laboratory. Namely *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Salmonella paratyphi*. The stock cultures were maintained in nutrient agar (NA) slant at 4°C and as the sub cultures. Working cultures were prepared by inoculating a loop full of each test microorganism in 3 ml of nutrient broth (NB). Broths were incubated at 37 °C for 24 hours. The suspension was diluted with sterile distilled water to obtain approximately 10^6 CFU/ml.

Antimicrobial screening

The antimicrobial screening was carried out by standard disc diffusion method and the compounds with more activity are subjected to separation using thin layer chromatography. Then the activities of separated compounds in TCL plates are studied using direct bioautography method.

Media Preparation and Disc Diffusion Method

Kirby-Bauer method was followed for disc diffusion assay[8]. In vitro antimicrobial activity was screened by using Nutrient Agar (NA) obtained from Hi-media. The NA plates were prepared by pouring 20 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 to 10 minutes and 0.1% inoculums suspension swabbed uniformly and the inoculums were allowed to stand. The extract with 3mg/disc concentration was loaded on 5mm sterile discs. The loaded discs were placed on the surface of medium and compound was allowed to diffuse and the plates were kept for incubation at 37 °C for 24hrs. Erythromycin (5µg/disc) was used as control. After 24 hours, the inhibition zone formed around the disc was measured with transparent ruler in millimetre. These studies were performed in triplicate.

Thin Layer Chromatography

Five solvents were used for thin layer chromatography in different ratios. The TLC plates were cut into the size of 10×5 (height × breath). Totally five samples were prepared, which showed best activity in disc diffusion method was chosen to run TLC. Hexane, Chloroform, Ethyl acetate, Acetone and Methanol were used in the ratios of Hexane: chloroform→ 9:1, 7:3, 5:5; Ethyl acetate: Methanol→ 9:1, 7:3, 5:5; Hexane: Chloroform: Ethyl acetate→6: 3: 1, 7: 2: 1 and Ethyl acetate: Acetone: Methanol→6: 3: 1, 7: 2: 1.

Rf Value

The location of each spot on the plate was represented

Table 3: Rf value of various extracts

S. NO.	Name of Solvent extract	Solvents and its ratio Hexane: Choloroform 7:3
1	AL_2	0.34,0.48, 0.55, 0.72, 0.85,0.89
2	CL_2	0.13,0.23,0.31,0.58,0.83,0.96
3	CF_3	5.4
S. NO.	Name of Solvent extract	Solvent and its ratio Ethyl acetate: Methanol 8:2
1	CF_3	0.64
S. NO.	Name of Solvent extract	Solvent and its ratio Hexane: chloroform: Ethyl aceate 7:2:1
1	AL_2	0.30, 0.37, 0.50, 0.58, 0.67,0.90, 0.96
2	CL_1	0.22, 0.41, 0.44, 0.61, 0.83, 0.91
S. NO.	Name of Solvent extract	Solvent and its ratio Hexane: chloroform: Ethyl aceate 6:3:1
1	CL_2	0.34,0.41,0.64,0.80, 0.93, 0.97

AL_2-*S.trilobactu(acetone)*, CL_1- *S. torvumleaf (hexane)* , CL_2- *S. torvum leaf (acetone),* CF_3-*S.torvum fruit(ethanol)* , Rf value of Bioactive molecule is highlighted in Yellow

numerically by calculating a Retention Factor (Rf). The RetentionFactor was calculated using the formula:

Retention factor = A / B

Where, A: distance travelled by solvent

B: distance travelled by solute

Agar Overlay Method

Agar overlay method was followed for Bioautography assay[9]. The agar overlay assay is a combination of contact and direct bioautography assay. In this method, the chromatogram is covered with molten agar medium. Agar was poured, solidification and incubated. The activity was observed using staining with tetrazolium dye and the inhibition one was visualized. The inoculation medium was prepared using 1.5 % Agar media and 1ml of culture medium was added to 20ml of agar medium. The developed TLC sheet was placed upon wet cotton on a petriplate. The inoculated agar medium was poured onto the TLC plate and incubated for 24hrs. After 24hours the inoculums was sprayed with p-INT and the zone formed was observed and compared with the developed chromatogram.

RESULTS

In current study antimicrobial properties were tested on three extracts via Hexane, Acetone and Ethanol of *Solanum xanthocarpum, Solanum trilobatum, Solanum indicum* leaves and fruits. Against eight bacteria's namely *Streptococcus pneumoniae, Streptococcus pyogenes, Salmonella paratyphi, Klebsiella pneumonia, Escherichia coli, Pseudomonas aerogenosa* and *Salmonella typhi*. The efficacy of the extracts was compared with the standard Erythromycin. Erythromycin was not able to inhibit all the bacterial species and the highest activity in control was observed against *Salmonella typhi* (35mm), lowest activity was observed against *E. coli* (9mm) and no activity was found against *Streptococcus pneumonia*.

Antimicrobial activity of S. trilobatum, S. xanthocarpum and S. indicum leaf extracts (table: 1)

All the three leaf extracts of *S. trilobatum, S. xanthocarpum* and *S. indicum* demonstrated antibacterial activity against all the microbes except *Klebsiella pneumonia* in *S. trilobatum* and *E. coli* in S. indicum. In *S. trilobatum* maximum activity of 13mm was recorded in hexane & acetone extracts against *Pseudomonas aeruginosa* and in *Streptococcus pyogenes*(acetone). Lowest activity of 9mm was observed in acetone extract against *Salmonella typhi* and *Salmonella paratyphi* respectively. In *S. xanthocarpum* maximum activity of 16mm was recorded in *Salmonella typhi* (hexane and ethanol), *Streptococcus pyogenes* (hexane). No bacteria is resistance to this plant extracts. Lowest inhibitory activity of 8mm was seen in ethanol extract against *Salmonella paratyphi.* In *S.indicum* maximum activity of 18mm was recorded in acetone extract against *Streptococcus pyogenes*, 18mm was recorded in ethanol extract against *Salmonella typhi.* Lowest inhibitory activity of 8mm was seen in acetone extract against *Klebsiella pneumonia* and *Pseudomonas aeruginosa* respectively.

Antimicrobial activity of S. trilobatum, S. xanthocarpum and S. indicum fruit extracts (table: 2)

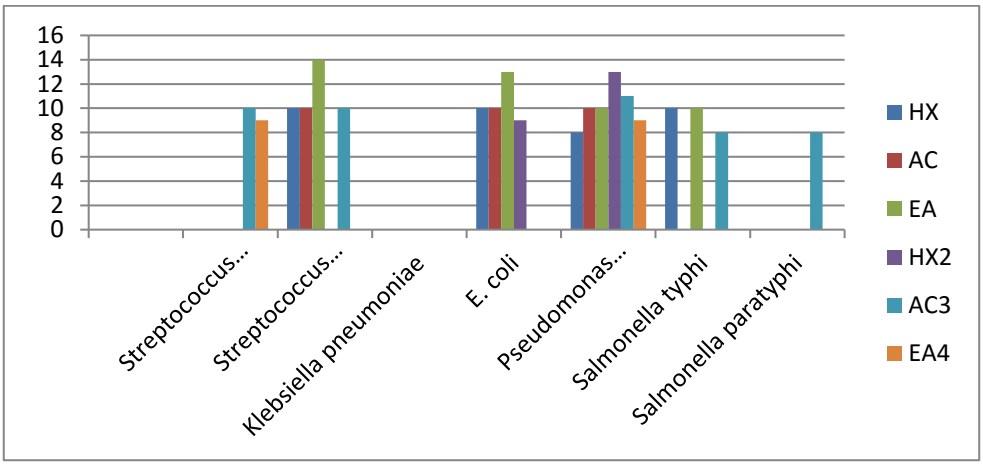
All the three fruit extracts of *S. trilobatum, S. xanthocarpum* and *S. indicum* demonstrated antibacterial activity against all the microbes except *Streptococcus pneumonia, Salmonella paratyphi* and *Klebsiella pneumonia* in *S. trilobatum*. *Klebsiella pneumonia* in *S. xanthocarpum* and *E. coli* in *S. indicum*. In *S. trilobatum* maximum activity of 14mm was recorded in ethanol extract against *Streptococcus pyogenes and E. coli.* Lowest inhibitory activity of 8mm was seen in hexane extract against *Pseudomonas aeruginosa.* In *S. xanthocarpum* maximum activity of 15mm was recorded in acetone against *Streptococcus pyogenes* and *Salmonella typhi* by ethanol extract. Lowest inhibitory activity of 8mm was observed in hexane extract against *E. coli.* In *S. indicum* maximum activity of 20mm was recorded in ethanol extract against *Streptococcus pyogenes, Salmonella typhi* and *Pseudomonas aeruginosa.* Lowest inhibitory activity of 8mm was seen in hexane extract against *Streptococcus pneumoniae.*

Thin Layer Chromatography

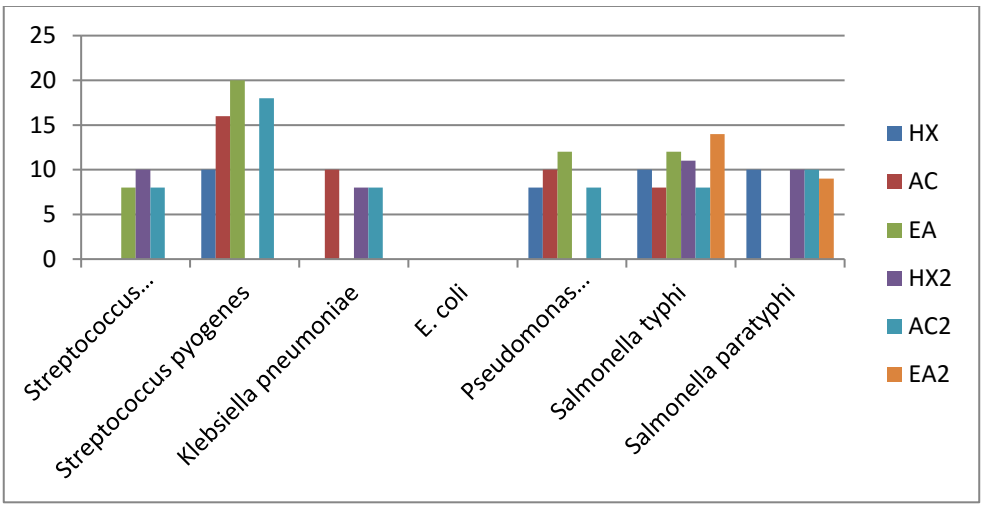
The bioactive molecule was separated by Thin Layer Chromatography method by employing organic solvents system. For this process four leaf extract of *S. trilobatum, S. xanthocarpum* and *S. indicum* and a fruit extract were chosen namely, *S. trilobatum leaf* (Hexane and Acetone extract) and *S. indicum leaf* (hexane and acetone) and fruit (ethanol).The Rf value was calculated and the results were tabulated(Table 4). Among the solvent systems hexane: chloroform system showed good separation of compounds.

Agar overlay Bioautography

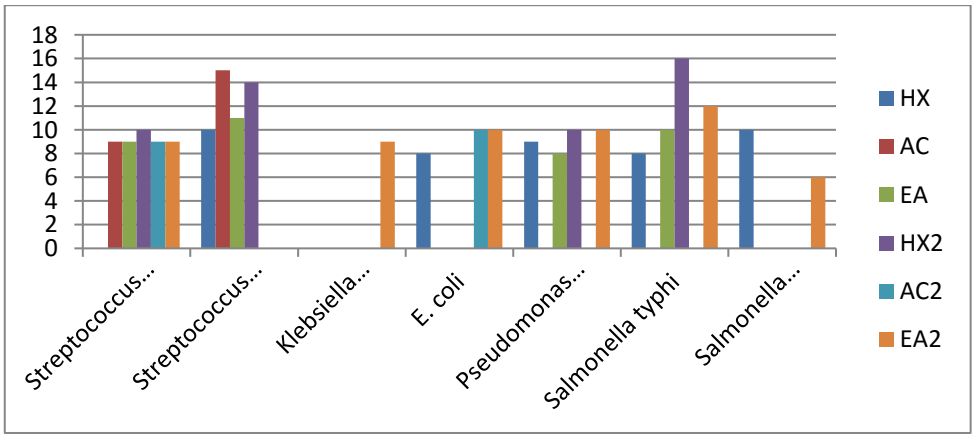
Three samples were selected based on the separation of compounds for bioautography. And these were tested against three bacterial organisms *Streptococcus pyogenes, Streptococcus pneumonia and Pseudomonas aeruginosa.* The inhibition zone was observed against *Streptococcus*



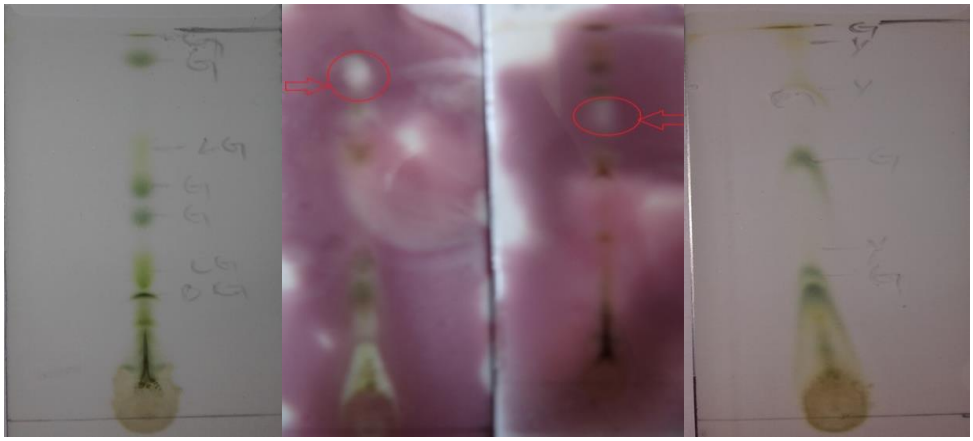
In-vitro Antimicrobial activity of *Solanum trilobatum* (Linn) Jack fruit and leave extracts.
HX: Hexane, AC: Acetone, EA: Ethanol- extract of fruit; HX2: Hexane, AC3: Acetone, EA4: Ethanol- extract of leave



In-vitro Antimicrobial activity of Solanum indium (Linn)Jack fruit and leave extracts
HX: Hexane, AC: Acetone, EA: Ethanol- extract of fruit; HX2: Hexane, AC2: Acetone, EA2: Ethanol- extract of leave



In-vitro Antimicrobial activity of *Solanum xanthocarpum* (Schard& H. wendl) Jack fruit and leave extracts
HX: Hexane, AC: Acetone, EA: Ethanol- extract of fruit; HX2: Hexane, AC2: Acetone, EA2: Ethanol- extract of leave.
Image showing separation through TLC and Agar overlay assay activity against *Streptococcus pyogenes*



[a] [b]
[c]
[a] TLC separation of *S. indicum* acetone leaf(CL_2) extract, [b] Agar overlay assay, [c] TLC separation of *S. xanthocarpum* acetone leaf(AL_2) extract.

pyogenes against *Solanum indicum* leaf extract (CL2) by the compound at Retention factor of 0.67 with a zone of 6mm, followed by *Solanum trilobatum* leaf extract (AL2) by the compound at Rf value 0.80 showing a zone of 4mm. No inhibition zone was observed against *Streptococcus pneumonia* and *Pseudomonas aeruginosa*.

DISCUSSION

The revival of interest on traditional medicines is mainly due to multidrug resistance in pathogens against modern synthetic drugs. Herbal medicines do not cause drug resistance against bacteria. The utilization of various indigenous medicinal plants is flourishing in different countries even today; with nearly 80% of rural population still depend on plant based medicines for primary health care[10]. In India, nearly 2500 species of herbal plants are used and 90% of them provide raw material for the herbal pharmaceuticals, which are collected from the wild habitats[11]. In the present study both leaves and fruit extracts of *S. trilobatum*, *S. xanthocarpum* and *S. indicum* showed antibacterial activities against almost all the tested organisms. Earlier investigation on antimicrobial activities of plant extract clearly showed that solvents like hexane, ethyl acetate, acetone, methanol and ethanol can extract active principles from plant parts[2]. In the present study, leaf extracts of *S. trilobatum* and *S. xanthocarpum* were more active than the fruit extracts; this might be due to the presence of maximum number of phytochemicals in leaves than fruits[12]. In *S. indicum* both the leaf and fruit extracts shows very good activities. Earlier study reported that ethanol and acetone extracts of plants had inhibited growth of many bacteria including *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*[13]. Same observation was reported by[2]. Ethanol extract of *S. trilobatum* leaves and fruit were found to be very effective against almost all the bacteria's and shows larger zone of inhibition (>10mm) against several organisms. Aqueous extract of *S. trilobatum* leaves showed antibacterial activity against tested bacterial strain *Klebsiella pneumonia* upto10 mm[12] whereas in the present study there was no inhibition zone observed in the solvent extracts. In the present study the ethanol extract of *Solanum indicum* inhibits a maximum zone of 20Mm in *Streptococcus pyogenes*, in acetone extract inhibits a maximum zone of 16Mm against *Streptococcus pyogenes.* Agar overlay technique is a hybrid of the two other methods and works successfully with a range of microorganism. The acetone extract of *S. indicum* and *S. trilobatum* showed activity against *Streptococcus pyogenes* in this method.

CONCLUSION

Solvent extract of *Solanum trilobatum*, *Solanum xanthocarpum* and *Solanum indicum* exhibited antimicrobial activity and inhibiting the growth of gram positive and gram negative bacteria at different degrees indicating the presence of broad spectrum antibiotics. Bioautography revealed the presence of specific and selective antimicrobial compounds in the extracts which may or may not have broad range activity. Further research needs to be done to extract and identify the active compounds. The active compound needs to be isolated and studied for its toxicity and *in vivo* efficacy resulting in development of better antimicrobial drugs.

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