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COMPARATIVE STUDY OF *OCIMUM BASILICUM* AND ITS *ENDOPHYTIC ACTINOMYCETES STREPTOMYCES FLAVOVIRIDIS* A3WK: EVALUATION OF ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITY

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ABSTRACT: Endophytic actinomycetes are the potential source for obtaining novel bioactive compounds. Present study evaluates the chemical constituents in *Ocimum basilicum* and its endophytic actinomycetes. Eleven endophytic actinomycetes were isolated from *O. basilicum* plant. Among eleven, one isolate was characterized by 16S rRNA and identified as *Streptomyces flavoviridis* A3WK. A comparative analysis of the FT-IR, GC-MS, antibacterial, antioxidant and anti-inflammatory properties of plant and endophytic actinomycetes extracts was conducted. 12 compounds each of *Streptomyces flavoviridis* A3WK and *O. basilicum* were identified by GC-MS. The FT-IR and GC-MS analysis reveals that *S. flavoviridis* A3WK has more antimicrobial, antioxidant activity and anti-inflammatory properties compared with the results of *O. basilicum*. Over all the activity of the endophytic actinomycetes was more compared to that of the *O. basilicum* extracts. The study reveals to show that the existence of endophytic actinomycetes may enhances medicinal value of the medicinal plants, and hence such studies help in the study of endophytic actinomycetes as well as their relevance in host plant.

INTRODUCTION: Endophytes are the micro-organisms that reside in the plant tissues without having any negative impact on the host plant¹. Endophytic actinomycetes are relatively unexplored as potential sources of novel natural products, and continue to draw the attention of researchers worldwide. Metabolic potential of such endophytic actinomycetes have been proved to be beneficial in the field of agriculture and medicine. Several secondary metabolites have been discovered from the endophytic actinomycetes from time to time².

Co-evolution made endophytes to adapt themselves to the niches and formed a completely compatible symbiont *via* gene transformation³. Due to factors shaping plant-endophyte interactions, some species of endophytes express different life styles, by ranging from mutualism through commensalism to parasitism⁴. The factors include genetic background, imbalance in nutrient exchange⁵ and environmental variations⁶. Medicinal plants play pivotal role in controlling human pathogen with their bioactive natural products.

However, natural habitats from medicinal plants have been threatened by over use and geopolitical instabilities⁷. So, it may become critical to develop alternative sources for medicinal plant products. Endophytes are chemical synthesizers inside plants, in other words they play the role as a selection system for microbes to produce bioactive

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substances with low toxicity towards higher organisms. Bioactive natural compounds produced by endophytes have been promising usefulness in medicinal plants². Majority of natural products produced by endophytic microorganisms showing antimicrobial activity can protect the host plant against phytopathogen⁸. The role of endophytes with respect to production of anticancer, antimicrobial, antioxidant and other biologically important compounds especially in medicinal plants provides a greater insight into the plants endophyte interaction and evolution of mutualism in medicinal plants⁹.

The endophytic microbes have been recognized to be fungi, bacteria and actinomycetes¹⁰. Number of secondary metabolites produced by endophytic actinomycetes is larger than that of any other endophytic microorganisms. Endophytic actinomycetes are considered as a rich and potential source of novel bioactive compounds and various bioactive compounds are continuously isolated from them¹¹⁻¹³. Endophytic actinomycetes, mainly from medicinal plants show the ability to inhibit or kill a wide variety of harmful microorganisms such as pathogenic bacteria, fungi and viruses. Thus, there is a great application value to develop antimicrobial drugs from endophytic actinomycetes, mainly from genus *Streptomyces*¹.

Ocimum basilicum L. (sweet basil) is a popular culinary herb belonging to the Lamiaceae family. It grows in several regions all over the world¹⁴. Basil is well known as a plant of a folk medicinal value and as such is accepted officially in a number of countries¹⁵. The leaves of basil are used in folk medicine as a tonic and vermifuge, and basil tea taken hot is good for treating nausea, flatulence, and dysentery¹⁶. Basil leaves possess strong antiviral, antimicrobial, antioxidant properties and it is also antibacterial in nature and thus acts as an insect repellent. Basil is used for treating stomach disorders such as intestinal gas, stomach spasms and loss of appetite. Basil is anti-inflammatory in nature due to the presence of eugenol, limonene and citronellol in the leaf. Basil leaf contains anti-aging properties that is capable of preventing aging harmful effects¹⁷.

The study aims to show that endophytic actinomycetes play a key role in attributing the

medicinal value to medicinal plants. A comparative study of endophytic actinomycetes and *Ocimum basilicum* was done and an attempt was made by FT-IR, GCMS, antioxidant activity and anti-inflammatory to show the relevance of endophytic actinomycetes in deciding the medicinal value of medicinal plants and this is the first report of such studies.

MATERIALS AND METHODS:

Samplings of Medicinal Plants: *Ocimum basilicum* (OB), indigenous to the region of north Karnataka, Dharwad, India were sampled over growing seasons (May to Feb). Dharwad situated on the edge of Western Ghats at an average altitude of 750 meters above sea level, is characterized by red and black soil and relatively high rainfall (average, 500 to 750mm). The experimental medicinal plants were sampled from botanical garden, Karnatak University, Dharwad. The plant was identified and authenticated by Dr. M. Jayaraj, Department of Botany, Karnatak University, Dharwad, Karnataka. Each sample was collected in a separate sterilized bag and tagged properly and processed in laboratory within 24 hours after sampling.

Isolation and Identification of Endophytic Actinomycetes: The plant samples were thoroughly washed under tap water to remove the soil debris. The inner tissue and the outer tissue of leaves, stem and root of the *O. basilicum* plants were carefully excised and subjected to surface sterilization. Sterilization was done with 10 min wash in 3.15% calcium hypochlorite, followed by a 15 min wash in 10% sodium hydrogen carbonate and a 2 min wash in 1% sodium azide. In each step, samples were rinsed with sterile distilled water. Surface sterilized tissue of *O. basilicum* plant was placed on the Starch casein agar (SCA) supplemented with cyclohexamide 50µg/mL and nystatin 50µg/mL and incubated for 3 - 4 weeks at 28 °C. Colonies on ISP-4 actinomycetes were sub cultured and purified on ISP and nutrient medium. The effectiveness of the surface sterilization was assessed by rinsing the samples with sterile distilled water and shaken for 30 sec at final step of procedure and cultured on the NA and kept overnight at 37 °C as a positive control¹⁸.

The isolates were picked from plates and purified on SCA and ISP-4 media and were tentatively

identified based on properties of colonies; the presence of aerial mycelium and substrate mycelium; spore mass color; distinctive diffusible pigmentation¹⁹. Based on phenotypic identification one representative of the isolated endophytic actinomycetes A3 was characterized by 16S rRNA.

The genomic DNA of endophytic actinomycetes A3 isolate was extracted by using a InstaGene™ matrix genomic DNA isolation kit catalog# 732-6030. This DNA was amplified by using the universal primer pair 27F-1492R using MJ research PTC-225 peltier thermal cycler, and PCR was carried out by adding 1µl of template DNA in 20µl of PCR reaction solution along with 27F/1492R primers for actinomycetes, under the following conditions, *i.e.* 35 amplification cycles at 94 °C for 45 sec, 55 °C, and 72 °C for 60 sec. The PCR product was sequenced using the 518F/800R primers (518F CCAGCAGCCGCGGTAATACG 800R TACCAGGGTATCTAATCC). Sequencing reactions were performed using a ABI PRISM® BigDye™ Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems). The phylogenetic tree aligned with the reference sequences was obtained from the nucleotide sequence database by using CUSTALW²⁰. A phylogenetic tree was constructed with MEGA software package by using neighbour - joining method.

Extraction of Bioactive Compounds from Endophytic Actinomycetes and *Ocimum basilicum* (OB): A purified isolate of endophytic actinomycetes A3 was grown on ISP-4 (International Streptomyces Project - 4) as a production media. The isolate was inoculated in ISP - 4 broth (Inorganic salt solution agar; soluble starch 10g / 500mL, K₂HPO₄ 1.0g MgSO₄.7H₂O 1.0g, NaCl 1.0g, (NH₄)₂SO₄ 2.0g, CaCO₃ 2.0g, Distilled water 500ml, Trace salts solution 1ml) and incubated at 28 °C in a shaker (180 rpm) for 7 days to allow optimum production of bioactive compound. The culture broth of endophytic actinomycetes was filtered to remove mycelium and extracted with ethyl acetate and was concentrated with a rotatory evaporators at room temperature²¹. Parallely *O. basilicum* plant was dried at 50 °C for 6h, pulverized into crumbs, and then extracted with ethyl acetate with slight modification and stored at 4 °C in dark brown bottle until further analysis²¹.

FT-IR Analysis of Endophytic Actinomycetes Extract and *O. basilicum* Extract: The functional groups of endophytic actinomycetes extract and plant extract were analyzed by using Fourier transform infrared spectroscopy (NICOLET 6700, USA) (FT-IR) and infrared spectra of the samples was recorded using KBr pellet technique. The molecular functional vibrations of chemical groups present in the sample were recorded at a resolution of 2 cm⁻¹ ranging from 4000–400 cm⁻¹.

GC-MS Analysis of Endophytic Actinomycetes Extract and *O. basilicum* Extract: The sample of endophytic actinomycetes extract and plant extract was analyzed by Gas Chromatograph - Mass Spectrometry (QP2010S, Shimadzu, Japan) (GC-MS). Interpretation of mass spectrum of GC-MS was made using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The molecular weight, structure and name of the test materials were ascertained²².

Antimicrobial Activity Assay of Endophytic Actinomycetes and *O. basilicum* Extract: The antimicrobial activity of the endophytic actinomycetes and *O. basilicum* extract were tested against Gram positive bacteria *Corneybacterium diphtheria* (ATCC75415) and *Streptococcus faecalis* ATCC47077, Gram negative bacteria *E. coli* ATCC25922, *Klebsiella pneumoniae* ATCC10031, *Salmonella thypi* ATCC700931. Test organisms were incubated in lysogeny broth (LB) for 20 h at 37 °C until the stationery phase was reached. Antimicrobial activity of endophytic actinomycetes and *O. basilicum* extract was evaluated by Disc diffusion paper - disc method with slight modification²³. Discs were dripped with an extracts, dried, and placed over the agar surface of plates freshly inoculated with test organisms. Suspensions of test organism were adjusted to 10⁶ cfu/mL. The plates were kept at room temperature for 2 h to allow the diffusion of extracts and then incubated at 37 °C for 24 h. The antimicrobial activity was measured by the diameter of the inhibition zone.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Ability Assay: The antioxidant

activity of endophytic actinomycetes and *O. basillicum* extracts was determined by the method of DPPH radical scavenging capacity with some modification²⁴. Different concentration (2, 4, 6, 8, 10mg/mL) of endophytic actinomycetes and *O. basillicum* extract were taken separately in the test tubes. Ascorbic acid was used as a reference standard. DPPH 0.1mM was freshly prepared in methanol and DPPH (2ml) was added to each tube containing different concentration of extracts (2ml). The reaction mixture was vortexed for 10s and then incubated in the dark for 30 min at room temperature. Ascorbic acid and methanol were used as positive control and blank, respectively. The absorbance of sample was measured at 517 nm. The DPPH radical scavenging activity was calculated by the following equation.

$$\% \text{ of DPPH radical scavenging activity} = \frac{[(\text{control Abs.} - \text{sample Abs.}) / \text{control Abs.}] \times 100}{1}$$

Where Abs control is the absorbance of the control reaction, Abs sample is the absorbance of the test sample. The experiments were carried out in triplicate and the data were expressed as average values.

Evaluation of *in vitro* Anti-inflammatory Activity: Anti-inflammatory activity of endophytic actinomycetes and *O. basillicum* extract was evaluated by protein denaturation method with slight modification²⁵. Anti-inflammatory drug Diclofenac sodium a strong non steroidal drug was used as a reference standard. About 2mL of different concentration of endophytic actinomycetes and *O. basillicum* extract (2-10mg/mL) or standard diclofenac sodium (10mg/mL) and 2.8mL of phosphate buffered saline (7.2) was mixed with 2mL of egg albumin and incubated at $(27 \pm 1)^\circ\text{C}$ for 15 mins. Keeping the reaction mixture at 70°C in a water bath for 10 min the denaturation was induced. After cooling, the absorbance was measured at 660nm by using double distilled water as a blank and control (PBS + Albumin). Each experiment was done in triplicate and average was taken. The percentage inhibition of protein denaturation was calculated by using the following formula.

$$\text{Inhibition \%} = \frac{[(\text{Control Abs.} - \text{Sample Abs.}) / \text{Control Abs.}] \times 100}{1}$$

Statistical Analysis: All experiment data were expressed as means \pm standard error. Statistical analyse of the data were performed using one-way ANOVA with SPSS version 16.0. Differences between means were located using Turkey's test ($P < 0.05$ and 0.01).

RESULTS:

Isolation and Identification of Endophytic Actinomycetes: The endophytic actinomycetes isolates were picked and purified on SCA and ISP-4 media. 11 endophytic actinomycetes isolates were detected and identified based on morphological criteria, colony characters, morphology of substrate and aerial hyphae, spores and pigment production as described by¹⁹. Among 11 isolates one isolate A3 as a representative has been characterized and identified by 16s RNA. The A3 isolate subjected to 16S rRNA gene sequence revealed the endophytes as *Streptomyces flavoviridis* A3WK. The percentage of 16S rRNA gene sequence similarities of these isolate to the closest type strain of NCBI database are presented in **Fig. 1**. The nucleotide sequences of *Streptomyces flavoviridis* A3WK obtained in this study was deposited in Gene Bank with accession number KP260508 as reported in our earlier studies¹⁸.

FT-IR Analysis of Ethyl Acetate Extract of *Streptomyces flavoviridis* A3WK and *O. basillicum*: The IR result of *S. flavoviridis* A3WK showed the broad spectrum range at 3444.13cm^{-1} which is assigned to the stretching vibration of phenol group and peak at 2966.31, 2921.76, 2857.39cm^{-1} is C-H vibration, 1638.41 refers to amines, 1480.52cm^{-1} refers to nitro compounds, 1391.30, 1326.54cm^{-1} refers to aromatic amines. The peak at 1105.74, 1023.31cm^{-1} indicates C-N stretching vibration of aliphatic amines, 861, and 801, 466 shows the presence of aromatics. The presence of phenols, carboxylic acid and alkanes functional group reveals that the extract of *Streptomyces flavoviridis*A3WK has antimicrobial activity and antioxidant activity **Fig 2**.

The IR results of *O. basillicum* showed that the broad spectrum range at 3440.43cm^{-1} corresponds to phenol group. The spectrum at 2925.26cm^{-1} and 2854.95cm^{-1} indicates the presences of carboxylic acid, 2128.77cm^{-1} refers to alkynes, 1741.40cm^{-1} refers to esters group, 1630.81cm^{-1} refers to amine

group, 1054.71 and 1158.73 cm^{-1} refers to alkyl halides. The FT-IR analysis revealed the presence of compounds which prove that there are more

characteristics of eugenol, naphthalenol and some halogens are present in these plant extracts **Fig. 2**.

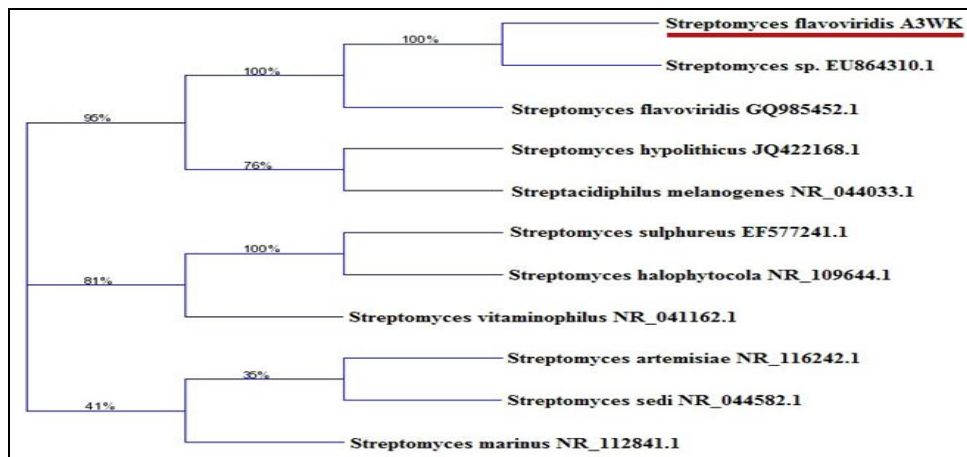


FIG. 1: PHYLOGENETIC TREE SHOWING THE RELATIONSHIPS OF ENDOPHYTIC *STREPTOMYCES FLAVOVIDIRIS* A3WK, RELATED SPECIES OF THE SAME GENUS AND OTHER TAXA BASED ON 16S rRNA GENE SEQUENCING

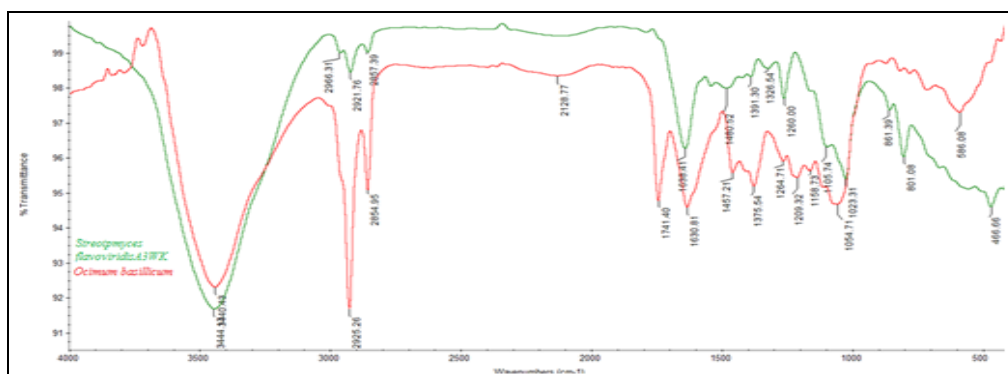


FIG. 2: FTIR ANALYSIS OF *STREPTOMYCES FLAVOVIDIRIS* A3WK AND *O. BASILLICUM*

Chemical Constituents of *Streptomyces flavoviridis* A3WK and *O. basillicum*: GC-MS was employed for analyzing the extracts of *Streptomyces flavoviridis* A3WK and *O. basillicum*. Identification of the components was done by comparison of their mass spectra with the NIST library and also based on the peak area, molecular mass and molecular

formula and was ascertained directly proportional to quantity of the compound present in the extract. Most of the identified compounds show various pharmaceutical applications. The detail of GC-MS is presented in **Fig. 3** and **4**.

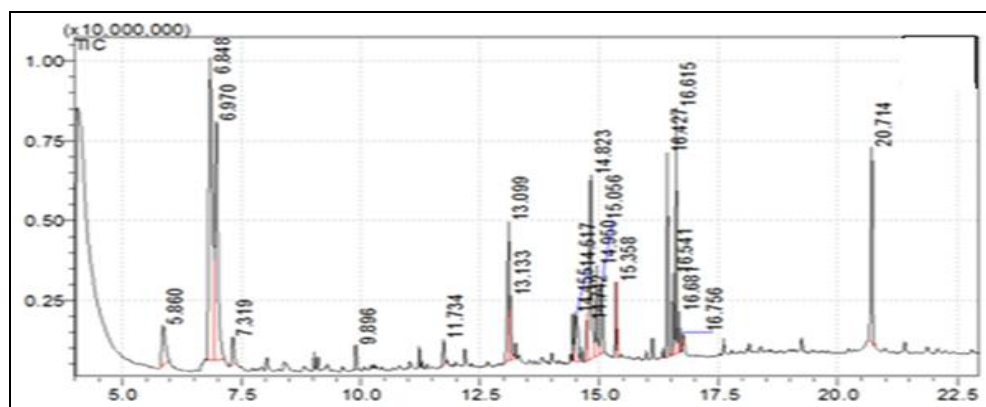
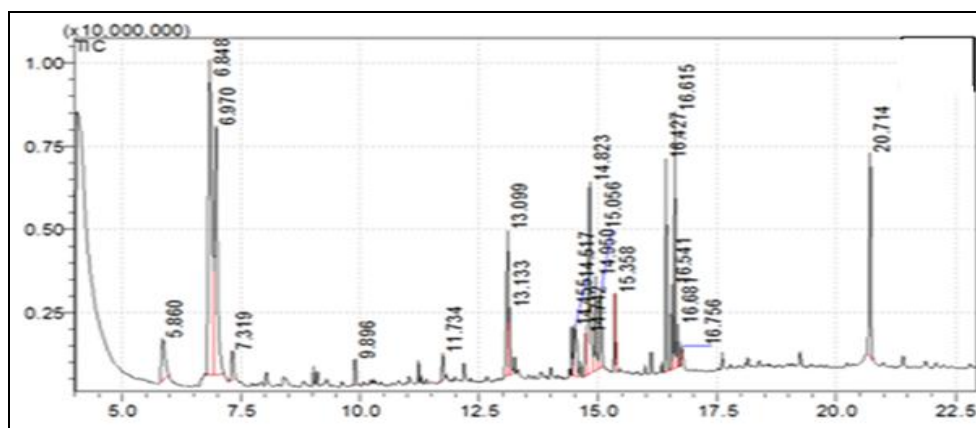


FIG. 3: GC - MS CHROMATOGRAM OF *STREPTOMYCES FLAVOVIDIRIS* A3WK

FIG. 4: GC - MS CHROMATOGRAM OF *O. BASILLICUM*

As shown in **Table 1**, 1H-Imidazole-4-carboxylic acid-2-methyl (48.19%) was the main constituent of the *Streptomyces flavoviridis* A3WK extract. Pharmaceutical studies revealed that 1H-Imidazole-4-carboxylic acid-2-methyl is highly potential antifungal agent²⁶. And along with this compound

it also produced other bioactive compounds such as n-Hexadecanoic acid, Pyrazol-5-carboxylic acid-3-methyl and Aziridine, 1-methyl. These compounds are used as an anti-inflammatory, antidiabetic, antibacterial and antitumor.

TABLE 1: CHEMICAL COMPOSITION OF *STREPTOMYCES FLAVOVIDIS* A3WK EXTRACT

Sl. No	Retention Time	Name of compound	Molecular formula	Molecular weight	Peak area%	Activity
1	4.668	Propionic acid, 3-(m-aminobenzoyl)-2-methyl-	C ₁₁ H ₁₃ NO ₃	207	0.68%	Antimicrobial agents ²⁸
2	5.215	Aziridine, 1-methyl	C ₃ H ₇ N	57	3.33%	Antitumor agent ²⁹
3	8.027	Pyrazol-5-carboxylic acid-3-methyl	C ₅ H ₆ N ₂ O ₂	126	2.91%	Antidiabetic and antibacterial ³⁰
4	9.392	1H-Imidazole-4-carboxylic acid-2-methyl	C ₅ H ₆ N ₂ O ₂	126	48.19%	Antifungal drug ²⁶
5	14.900	Trifluoroacetylpentadecane	C ₁₅ H ₂₇ F ₃ O ₂	296	0.76%	Antimicrobial agent ³¹
6	15.085	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	14.19%	Anti inflammatory ³²
7	15.283	1H-pyrazole-3-carboxylic acid-5-methyl	C ₇ H ₁₀ N ₂ O ₂	154	1.25%	Antioxidant and antimicrobial ³³
8	16.417	2, 6-octadiene, 2, 6-dimethyl	C ₁₀ H ₁₈	138	0.29%	Antimicrobial agent ³⁴
9	16.755	2 nitro phenol	C ₆ H ₅ NO ₃	312	4.99%	Fungicide ³⁵
10	16.790	1H-Imidazole, 2-methyl-1-propyl	C ₇ H ₁₂ N ₂	124	4.96%	Antifungal activity ³⁶
11	16.952	Glutraldehyde(4methyl pentanal)	C ₆ H ₁₂ O	129	15.98%	Sterilizing and disinfectant agent ³⁷
12	18.493	1Methyl-3-nitro-5[4-nitropyrazole-1-yl]	C ₆ H ₅ N ₇ O ₄	239	2.54%	Antimicrobial and antitumor ³⁸

As shown in **Table 2**, Thiomorpholin (22.48%) was the main constituent of the extract of *O. basillicum* and has anti hyperglycemic activity which inhibits α -amylase and α -glucosidase²⁷. Various pharmaceutical compounds were also identified, such as eugenol, 1-Naphthalenol, 3-Chloro-tetrahydrofuran-2-Carbonitrile these are the main constituent of *O. basillicum*, and that are used as an anesthetic agent, insecticidal agent and antiarrhythmic agent.

Antibacterial Activity: The antimicrobial activity of *Streptomyces flavoviridis* A3WK and *O. basillicum* was evaluated using disc-diffusion method. Three gram negative bacteria (*E. coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031, *Salmonella thypi* ATCC 700931) and two highly pathogen Gram positive bacteria (*Corneybacterium diphtheria* (ATCC 75415) and *Streptococcus faecalis* ATCC 47077) was used for testing antibacterial activities. *Streptomyces flavoviridis* A3WK in comparison

with *O. basillicum* harbored significant inhibitory activity against both Gram negative and Gram positive bacteria at $40\mu\text{g mL}^{-1}$ as shown in **Table 3**.

TABLE 2: CHEMICAL COMPOSITION OF *O. BASILLICUM* EXTRACT

S. no.	Retention Time	Name of Compound	Molecular Formula	Molecular weight	Peak area%	Activity
1	5.860	2-propenoic acid, 3 phenyl, methyl ester	$\text{C}_{10}\text{H}_{10}\text{O}_2$	162	3.15%	Cholesterol lowering ²⁸
2	6.848	Thiomorpholine	$\text{C}_4\text{H}_9\text{NS}$	103	22.48%	Antihyperglycemic agents ²⁷
3	7.319	Eugenol	$\text{C}_{10}\text{H}_{12}\text{O}_2$	178	1.44%	Anesthetic agents ³⁹
4	9.896	4- phenyl piperidine	$\text{C}_{11}\text{H}_{15}\text{N}$	161	0.81%	Antidepressant agents ⁴⁰
5	13.133	n-Hexadeconic acid	$\text{C}_{16}\text{H}_{32}\text{O}_2$	256	1.92%	Anti inflammatory agents ³²
6	14.517	1H-Indole-4-carboxaldehyde	$\text{C}_9\text{H}_7\text{NO}$	145	2.74%	Antioxidant agent ⁴¹
7	14.823	1-Naphthalenol	$\text{C}_{15}\text{H}_{10}\text{O}$	202	9.54%	Insecticide ⁴²
8	15.056	Glutraldehyde(4methyl pentanal)	$\text{C}_6\text{H}_{12}\text{O}$	129	2.74%	Sterilizing and disinfectant agent ³⁷
9	15.349	Alpha- Bisabolol acetate	$\text{C}_{15}\text{H}_{28}\text{O}_2$	264	0.54%	Antiaging property ⁴³
10	16.428	3-Chloro-tetrahydrofuran-2-Carbonitrile	$\text{C}_5\text{H}_6\text{C}_1\text{NO}$	131	5.95%	Antiarrhythmic agent ⁴⁴
11	16.681	4-Chlorobenzenesulfonamide, N-methyl	$\text{C}_7\text{H}_8\text{ClNO}_2\text{S}$	205	1.08%	Antibacterial agent ⁴⁵
12	20.714	2-amino benzaldehyde	$\text{C}_7\text{H}_7\text{NO}$	121	7.42%	Fragrance agent ⁴⁶

Earlier study demonstrates that ampicillin is effective against *Corneycbacterium diphtheria*, showing that it had developed ampicillin resistance⁴⁷. It is interesting to note that ampicillin as a positive control showed less activity against *Corneycbacterium diphtheria* than that of *Streptomyces flavoviridis* A3WK. However, this clinical isolate was significantly inhibited by *Streptomyces flavoviridis* A3WK than its host **Table 3**.

TABLE 3: ANTIBACTERIAL ACTIVITY OF *STREPTOMYCES FLAVOVIRIDIS* A3WK AND *O. BASILLICUM* EVALUATED BY DISC DIFFUSION METHOD

Test organisms	Inhibition zone (mm)		
	<i>Streptomyces flavoviridis</i> A3WK ($40\mu\text{g mL}^{-1}$)	<i>O. basillicum</i> ($40\mu\text{g mL}^{-1}$)	Ampicillin ($40\mu\text{g mL}^{-1}$)
<i>Klebsiella pneumonia</i> ATCC 10031 (-)	18	5	22
<i>Corneycbacterium diphtheria</i> ATCC 75415(+)	19	5	15
<i>Salmonella parathypi</i> ATCC 700931 (-)	9	4	11
<i>Streptococcus faecalis</i> ATCC 47077 (+)	9	2	21
<i>E. coli</i> ATCC 25922(-)	8	4	9

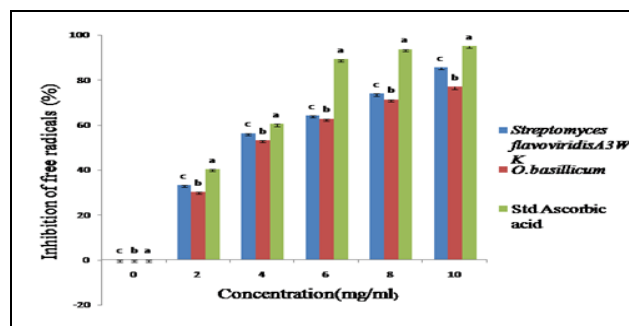
Antioxidant Activity: In the present study, the antioxidant activity of extracts of *Streptomyces flavoviridis* A3WK and *O. basillicum* was compared with standard antioxidant compound i.e. ascorbic acid to assay the radical scavenging ability of both extracts by DPPH method. The result is shown in **Table 4** and **Fig. 5**.

picrylhydrazyl (DPPH) method has been widely used to asses antioxidant assay of herbs and their endophytic extract⁴⁸.

TABLE 4: DPPH RADICAL SCAVENGING ACTIVITY OF *STREPTOMYCES FLAVOVIRIDIS* A3WK AND *O. BASILLICUM* WITH STANDARD ASCORBIC ACID

Concentration (mg/mL)	Inhibition		
	Standard Ascorbic acid** (10mg/mL)	<i>Streptomyces flavoviridis</i> A3WK extract*	<i>Ocimum basillicum</i> extract
0	0	0	0
2	40.37±0.0025	33.26±0.0020	30.23±0.0015
4	60.43±0.0030	56.37±0.0026	53.16±0.004
6	89.16±0.002	64.24±0.0005	62.80±0.0023
8	93.72±0.0011	73.94±0.001	71.35±0.0020
10	94.98±0.0011	85.61±0.001	77.00±0.0045

**correlation is significant at 0.01 levels *correlation is significant at 0.05 levels values are means ± SD, n = 3. Results were analyzed using one-way ANOVA

**FIG. 5: COMPARISON OF *STREPTOMYCES FLAVOVIRIDIS* A3WK AND *O. BASILLICUM* WITH STANDARD ASCORBIC ACID FOR RADICAL SCAVENGING ACTIVITY BY DPPH METHOD. A - STD ASCORBIC ACID, B - *OCIMUM BASILLICUM*, C - *STREPTOMYCES FLAVOVIRIDIS* A3WK**

The antioxidant activities of both the extracts and standard ascorbic acid (positive control) were not significantly different, as ascorbic acid yielded 94.98%, were as *Streptomyces flavoviridis* A3WK gave 85.61% and *O. basillicum* 77.00%. Percentage inhibition of the results showed that both the extracts have antioxidant activity but *Streptomyces flavoviridis* A3WK has more antioxidant activity in comparison to its host *O. basillicum*.

Anti-inflammatory Activity: In the present study, anti-inflammatory activity of *S. flavoviridis* A3WK and *O. basillicum* were done by protein denaturation method. The anti-inflammatory activities of both the extracts were compared with standard diclofenac sodium as reference drug²⁵. The anti-inflammatory activity of endophytic extract was significantly more compared to that of its host plant *O. basillicum*. At 10mg/mL standard drug showed 93.51% inhibitory activity and *Streptomyces flavoviridis* A3WK and *O. basillicum* showed 72.23% and 59.27% respectively **Table 5**.

TABLE 5: IN VITRO ANTI-INFLAMMATORY ACTIVITY OF STREPTOMYCES FLAVOVIRIDIS A3WK AND O. BASILLICUM

Extract	Concentration	Inhibition
<i>Streptomyces flavoviridis</i> A3WK extract	10mg/mL	72.23±0.0015
<i>Ocimum basillicum</i> extract	10mg/mL	59.27±0.079
Diclofenac sodium	10mg/mL	93.44±0.0041

Values are means ± SD, n = 3. Results were analyzed using one-way ANOVA.

DISCUSSION: Microbial endophytes of medicinal plants participate in the metabolic pathways of medicinal plants and produce analogous or novel bioactive secondary metabolites⁴⁹. Endophytic actinomycetes as one of the substantial residents in plant tissues is being considered as an important area of research which is rarely studied and there is an ever increasing need for novel organisms and their bioactive products⁵⁰. *Ocimum basillicum* is used traditionally as a medicinal plant and is used for various healing properties and potential therapeutic properties such as antiviral, anti-inflammatory, antioxidant, anti-hypertensive, antiarrhythmic, antidepressant and larvicidal effects⁵¹, but the studies on the antibacterial, anti-inflammatory, antioxidant from its endophytes have been neglected. This study is the first report of isolating *Streptomyces flavoviridis* A3WK as

endophytic actinomycetes from *Ocimum basillicum*.

The results obtained from the comparative analysis of medicinal plant and its endophytic actinomycetes indicates that among 11 endophytic actinomycetes isolates, one isolate was characterized based on their potentiality and identified by 16S rRNA as *Streptomyces flavoviridis* A3WK as reported in our earlier studies¹⁸. FTIR results were confirmed by the Narendharn S *et al.*,⁵² who revealed that the presence of functional group, phenols, carboxylic acid and alkanes shows that the FTIR vibration spectrum has antimicrobial and antioxidant properties. Parallely *Ocimum basillicum* FTIR were in strong agreement with⁵³ who revealed that the FTIR spectra shows the eugenol, naphthalenol and some halogens are present in the *Ocimum basillicum* extract. The bioactivity of extract of *Streptomyces flavoviridis* A3WK had excellent antibacterial activity against hospital pathogens comparing with that of its host *Ocimum basillicum*.

Thus, it is evident that antibiotic originating from endophytic actinomycetes recovered from medicinal plants is found to be having strong and broad spectrum microbicidal activity¹.

Earlier studies had shown that endophytes can produce the same or similar secondary metabolites as their host. Camptothecin a bioactive compound co-produced by the plants as well as their associated endophytes which has anticancer property⁵⁴. Similarly, in the present study it is found that *Streptomyces flavoviridis* A3WK and its host *O. basillicum* produced n-Hexadecanoic acid, an anti-inflammatory agent.

Hence in the present study simple and viable protein denaturation bioassay method was used to evaluate the anti-inflammatory activity of plant and endophyte extracts with diclofenac sodium as reference drug. Interestingly, in comparison to the reference drug, *Streptomyces flavoviridis* A3WK extract showed higher inhibitory activity (72.23 ± 0.0015%) whereas plant extract exhibited moderate activity (**Table 5**).

The results showed more anti-inflammatory activity by endophyte than its host though the n-hexadecanoic acid was detected from host as well

as *Streptomyces flavoviridis* A3WK. And in GCMS profile, the relative abundance of the compound was high in *Streptomyces flavoviridis* A3WK extract as compared to that of the plant, hinting abundant bioactive compounds produced by endophytes.

One of the best and successful facts of contribution of more bioactive compounds from endophyte than its host is the multibillion-dollar anticancer drug Taxol. The compound initially was isolated from pacific yew tree, *Taxus brevifolia*⁵⁵; these plants are slow - growing with generally isolated geographical distribution.

Investigations revealed endophytes are responsible for producing the exact same compound⁵⁶. Presence of n-hexadecanoic acid was important in knowing the biology and co-evolutionary relationship between endophytes and its host *O. basillicum*. This makes real possibility that genes involved in natural products biosynthesis could be exchanged *via* horizontal gene transfer (HGT) between microbes and plants, resulting in production of plant- derived compounds by microbes⁵⁷.

1H- Indole- 4- carboxyaldehde an antioxidant product was detected in the plant extract of *O. basillicum* and 1H-pyrazole - 3- carboxylic acid-5-methyl was detected in endophyte extract, interestingly exhibited antioxidant and antimicrobial property. According to Atsuko Matsumoto *et al.*, 2017 natural bioactive metabolites with multifold applications expand endophytic actinomycetes born compounds and their hosts; a correlation seems to exist between their ecological basis and bioactive metabolites⁵⁸.

The correlated study of metabolite profile of both plant *O. basillicum* and endophyte *S. flavoviridis* A3WK through various parameters reveals the phytochemical properties like antioxidant, anti-inflammatory and antibacterial, but it might be a possibility that the metabolite detected in plant might be contributed by endophytes; while compounds like 2-propenoic acid, 3 phenyl, methyl ester used for lowering cholesterol, thiomorpholine have antihyperglycemic activity; the anesthetic property of *O. basillicum* is due to the presences of Eugenol; 4-phenyl piperidine has antidepressant

activity; 1-naphthalenol has insecticide activity; alpha - bisabolol acetate this compound has antiaging property; 3- Chloro-tetrahydrofuran- 2- Carbonitrile has antiarrhythmic agent; 4-Chloro-benzenesulfonamide, N-methyl has antibacterial activity, and 2-amino benzaldehyde is used as a fragrance agent, were detected exclusively in the plant extract of *O. basillicum* and the medicinal properties of these detected compounds are naturally present in *O. basillicum* plant²¹.

While aziridine, 1-methyl which has antitumor activity, pyrazol-5-carboxylic acid-3-methyl has both antidiabetic and antibacterial activity, tri-fluoroacetoxy pentadecane; propionic acid, 3-(m-aminobenzoyl)-2-methyl-; 2,6-octadiene,2,6-dimethyl which has antimicrobial property; 1H-Imidazole-4-carboxylic acid-2-methyl; 2 nitro phenol; 1H-Imidazole, 2-methyl-1-propyl that has antifungal property, 1Methyl- 3- nitro- 5[4-nitropyrazole-1-yl] which has dual function of antimicrobial and antitumor activity.

These findings suggest the presence of these compounds enhances the medicinal property of medicinal plants. This study results are in confirmation with findings of Silva reports that the endophytic actinomycetes crude extract is highly active and found to show antibacterial, antifungal, antiviral, anti-inflammatory and antitumor compounds⁵⁹.

In the present study, antioxidant activity of *O. basillicum* and *Streptomyces flavoviridis* A3WK extracts was evaluated by DPPH free radical scavenging assay compared with standard ascorbic acid. This is an easy and widely used method for testing *in vitro* antioxidant activity of natural compounds or plant extracts⁶⁰. Comparing with standard *Streptomyces flavoviridis* A3WK showed higher activity than its host *O. basillicum* (85.61 ± 0.001). Tanvir studies showed that endophytic actinomycetes can be better antioxidant than the plants⁶¹. Similarly X liu M reported that endophytic actinomycetes can be a potential source of novel natural antioxidant in comparison with medicinal plants, fruits and vegetables though they are naturally present as a natural antioxidant⁶². From this it is evident that endophytic actinomycetes could be a promising source of

antimicrobial and antioxidant activity which gives medicinal value to the medicinal plant.

In spite of the uncertainty of the bioactive compounds produced by the endophytes unique to it, but not produced by the plant; confirms protection for the plants from many plant pathogens which facilitates to study the role of these endophytic microbes and their role in the plant¹⁰.

CONCLUSION: This study an initiation to emphasize the role of endophytic actinomycetes in determining the medicinal value to the plants where they reside and metabolite profile of both host plant and endophytic actinomycetes, contributed phytopharmacological compounds attributed to various photochemical properties like antioxidant, anti-inflammatory and antibacterial. *Ocimum* is used for treatment of several ailments like antiarrhythmic agent, lowering blood sugar level, anti-inflammatory, lowering cholesterol, anti-depressant agent *etc.* The GC MS profiling clearly shows the evidence of role of endophytic actinomycetes *Streptomyces flavoviridis* A3WK showed various biological properties, which highlighted its importance as potential pharmacological agents. Future research at the molecular level can reveal plant - microbe interaction which can be explored for bio-prospecting of potential bioactive compounds in medicinal plants

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