IJPSR (2018), Volume 9, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 June, 2017; received in revised form, 05 August, 2017; accepted, 11 August, 2017; published 01 March, 2018

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

Waheeda Khanam and Shyam Kumar Vootla*

Department of Microbiology and Biotechnology, Karnatak University, Dharwad - 580003, Karnataka, India.

Keywords:

Endophytic actinomycetes, Medicinal plants, GC/MS, FT-IR, Antioxidant activity, Anti-inflammatory activity

Correspondence to Author:
Prof. Shyam Kumar Vootla

Professor,
Department of Microbiology and Biotechnology, Karnatak University, Dharwad - 580003, Karnataka, India.

E-mail: vootlashyam@kud.ac.in

ABSTRACT: Endophytic actinomycetes are the potential source for obtaining novel bioactive compounds. Present study evaluates the chemical constituents in *Ocimum basillicum* and its endophytic actinomycetes. Eleven endophytic actinomycetes were isolated from *O. basillicum* 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. basillicum* 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. basillicum*. Over all the activity of the endophytic actinomycetes was more compared to that of the *O. basillicum* 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 plant [1]. 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 time [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(3).1023-34

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(3).1023-34

Co-evolution made endophytes to adapt themselves to the niches and formed a completely compatible symbiont *via* gene transformation [3]. Due to factors shaping plant-endophyte interactions, some species of endophytes express different life styles, by ranging from mutualism through commensalism to parasitism [4]. The factors include genetic back-ground, imbalance in nutrient exchange [5] and environmental variations [6]. 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 instabilities [7]. 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

substances with low toxicity towards higher organisms. Bioactive natural compounds produced by endophytes have been promising usefulness in medicinal plants [2]. Majority of natural products produced by endophytic microorganisms showing antimicrobial activity can protect the host plant against phytopathogen [8]. 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 plants [9].

The endophytic microbes have been recognized to be fungi, bacteria and actinomycetes [10]. 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 them [11 - 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 basillicum L. (sweet basil) is a popular culinary herb belonging to the Lamiaceae family. It grows in several regions all over the world [14]. Basil is well known as a plant of a folk medicinal value and as such is accepted officially in a number of countries [15]. 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 dysentery [16]. 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 effects [17].

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 basillicum* 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 basillicum* (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. basillicum* 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. basillicum* 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 control [18].

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 pigmentation [19]. 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® BigDyeTM 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 CUSTALW [20]. 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, K_2HPO_4 1.0g $MgSo_4.7H_2O$ 1.0g, NaCl 1.0g, $(NH_4)_2SO_4$ 2.0g, $CaCO_3$ 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 temperature [21]. Parallely *O. basillicum* 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 analysis [21].

FT-IR Analysis of Endophytic Actinomycetes Extract and *O. basillicum* 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 ascertained [22].

Antimicrobial Activity Assay of Endophytic Actinomycetes and *O. basillicum* Extract: The antimicrobial activity of the endophytic actinomycetes and *O. basillicum* 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. basillicum* extract was evaluated by Disc diffusion paper - disc method with slight modification [23]. 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 to10^6 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 modification [24]. 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.

% of DPPH radical scavenging activity = [(control Abs. - sample Abs.)/ control Abs.] × 100

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 modification [25]. 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 ± 1) °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.

Inhibition % = [(Control Abs. - Sample Abs.) / Control Abs.] × 100

Statistical Analysis: All experiment data were expressed as means ± 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 by [19]. 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 studies [18].

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, 1480.52 cm^{-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 1158.73cm^{-1} refers to alkyl halides. The FT-IR analysis revealed the presence of compounds which prove that there are more characteristics of eugenol, naphtalenol and some halogens are present in these plant extracts **Fig. 2**.

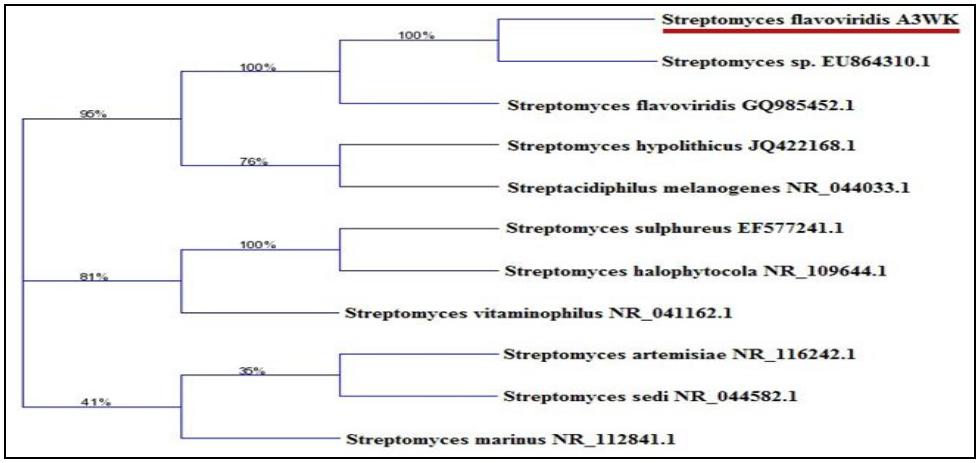

FIG. 1: PHYLOGENETIC TREE SHOWING THE RELATIONSHIPS OF ENDOPHYTIC *STREPTOMYCES FLAVOVIRIDIS* A3WK, RELATED SPECIES OF THE SAME GENUS AND OTHER TAXA BASED ON 16S rRNA GENE SEQUENCING

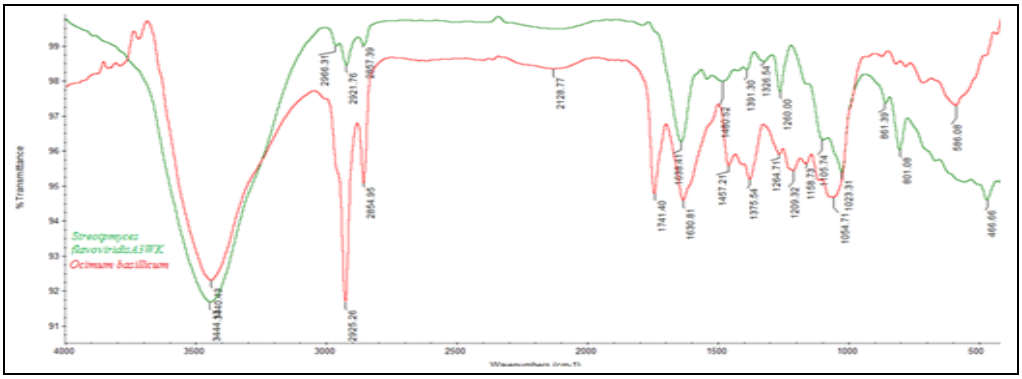

FIG. 2: FTIR ANALYSIS OF *STREPTOMYCES FLAVOVIRIDIS* A3WK AND *O. BASILLICUM*

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**.

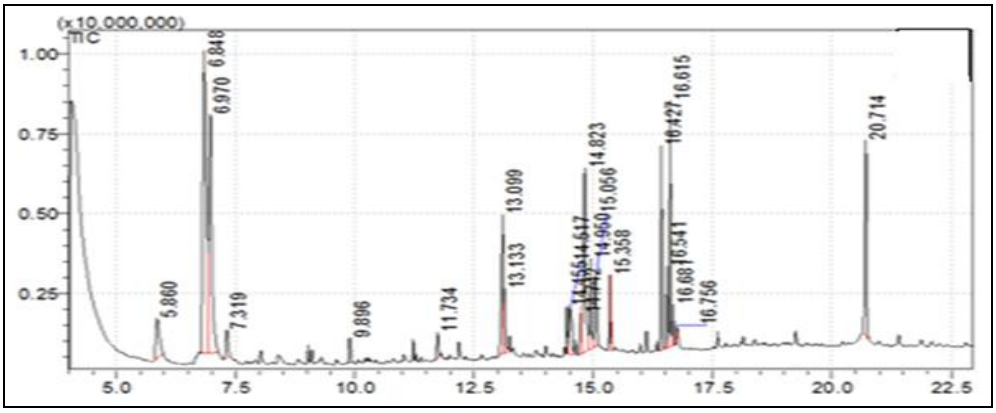

FIG. 3: GC - MS CHROMATOGRAM OF *STREPTOMYCES FLAVOVIRIDIS* A3WK

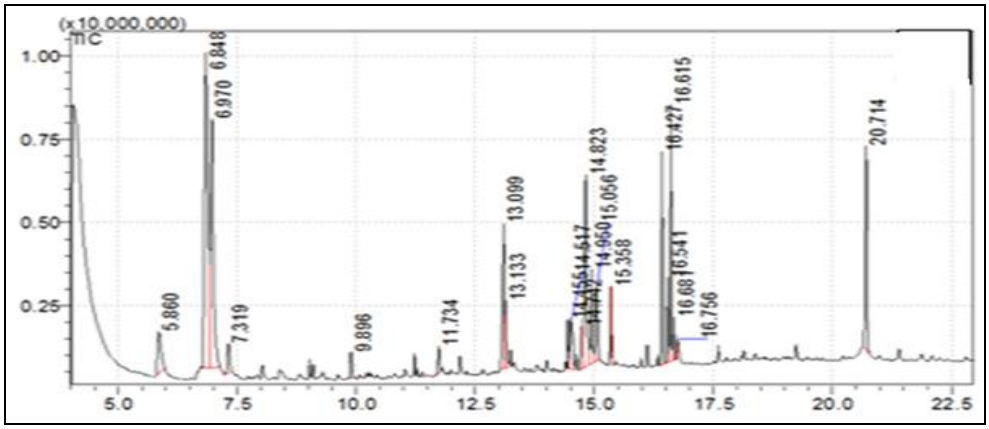

FIG. 4: GC - MS CHROMATOGRAM OF *O. BASILLICUM*

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 agent [26]. 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 FLAVOVIRIDIS* 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_3H_7N	57	3.33%	Antitumor agent [29]
3	8.027	Pyrazol-5-carboxylic acid-3-methyl	$C_5H_6N_2O_2$	126	2.91%	Antidiabetic and antibacterial [30]
4	9.392	1H-Imidazole-4-carboxylic acid-2-methyl	$C_5H_6N_2O_2$	126	48.19%	Antifungal drug [26]
5	14.900	Trifluroacetoxypentadecane	$C_{15}H_{27}F_3O_2$	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	$C_7H_{10}N_2O_2$	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	$C_6H_5NO_3$	312	4.99%	Fungicide [35]
10	16.790	1H-Imidazole, 2-methyl-1-propyl	$C_7H_{12}N_2$	124	4.96%	Antifungal activity [36]
11	16.952	Glutraldehyde(4methyl pentanal)	$C_6H_{12}O$	129	15.98%	Sterilizing and disinfectant agent [37]
12	18.493	1Methyl-3-nitro-5[4-nitropyrazole-1-yl]	$C_6H_5N_7O_4$	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-glucosidase [27]. 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 40µgmL^{-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	$C_{10}H_{10}O_2$	162	3.15%	Cholesterol lowering [28]
2	6.848	Thiomorpholine	C_4H_9NS	103	22.48%	Antihyperglycemic agents [27]
3	7.319	Eugenol	$C_{10}H_{12}O_2$	178	1.44%	Anesthetic agents [39]
4	9.896	4- phenyl piperidine	$C_{11}H_{15}N$	161	0.81%	Antidepressant agents [40]
5	13.133	n-Hexadeconic acid	$C_{16}H_{32}O_2$	256	1.92%	Anti inflammatory agents [32]
6	14.517	1H-Indole-4-carboxaldehyde	C_9H_7NO	145	2.74%	Antioxidant agent [41]
7	14.823	1-Naphthalenol	$C_{15}H_{26}O$	222	9.54%	Insecticide [42]
8	15.056	Glutraldehyde(4methyl pentanal)	$C_6H_{12}O$	129	2.74%	Sterilizing and disinfectant agent [37]
9	15.349	Alpha- Bisabolol acetate	$C_{15}H_{28}O_2$	264	0.54%	Antiaging property [43]
10	16.428	3-Chloro-tetrahydrofuran-2-Carbonitrile	$C_5H_6C_1NO$	131	5.95%	Antiarrhythmic agent [44]
11	16.681	4-Chlorobenzenesulfonamide, N-methyl	$C_7H_8ClNO_2S$	205	1.08%	Antibacterial agent [45]
12	20.714	2-amino benzaldehyde	C_7H_7NO	121	7.42%	Fragrance agent [46]

Earlier study demonstrates that ampicillin is effective against *Corneybacterium diphtheria*, showing that it had developed ampicillin resistance [47]. It is interesting to note that ampicillin as a positive control showed less activity against *Corneybacterium 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)		
	Sterptomyces flavoviridis A3WK (40µg mL^{-1})	*O. basillicum* (40µg mL^{-1})	Ampicillin (40µg mL^{-1})
Klebsiella pneumonia ATCC 10031 (-)	18	5	22
Corneybacterium diphtheria ATCC 75415(+)	19	5	15
Salmonella parathypi ATCC 700931 (-)	9	4	11
Streptococcus faecalis ATCC 47077 (+)	9	2	21
E. coli 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**. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) method has been widely used to asses antioxidant assay of herbs and their endophytic extract [48].

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)	*Streptomyces flavoviridis* A3WK extract*	*Ocimum basillicum* 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

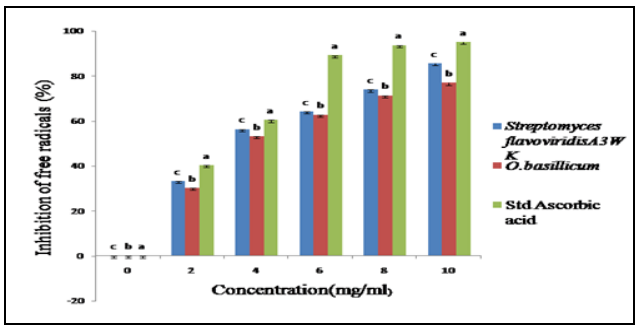

FIG. 5: COMPARISON OF *STREPTOMYCES FLAVOVIRIDIS* A3WK AND *O. BASILLICUM* WITH STANDARD ASCORBIC ACID FOR RADICAL SCAVENGING ACTIVITY BY DPPH METHOD. A - STD ASCORBIC ACID, B - *OCIMUM BASILLICUM*, C - *STREPTOMYCES FLAVOVIRIDIS* A3WK

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 drug [25]. 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
Streptomyces flavoviridis A3WK extract	10mg/mL	72.23±0.0015
Ocimum basillicum 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 metabolites [49]. 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 products [50]. *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 effects [51], but the studies on the antibacterial, anti-inflammatory, antioxidant from its endophytes have been neglected. This study is the first report of isolating *Streptomyces flavovirdis* 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 flavovirdis* A3WK as reported in our earlier studies [18]. 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 with [53] 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 flavovirdis* 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 activity [1].

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 property [54]. Similarly, in the present study it is found that *Streptomyces flavovirdis* 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 flavovirdis* 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 compound [56]. 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 microbes [57].

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 metabolites [58].

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* plant [21].

While aziridine, 1-methyl which has antitumor activity, pyrazol-5-carboxylic acid-3-methyl has both antidiabetic and antibacterial activity, tri-fluroacetoxypentadecane; 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 compounds [59].

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 extracts [60]. Comparing with standard *Streptomyces flavovirdis* 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 plants [61]. 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 antioxidant [62]. 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 plant [10].

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 phytopharmocological 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

ACKNOWLEDGEMENT: Author Waheeda Khanam grateful to University Grants Commission New Delhi, for providing Research Fellowship for PhD through University of Potential Excellence (UPE) Scheme (No.KU/Sch/UGC-UPE/2013-14/ 109) and USIC Department for providing timely help.

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

REFERENCES:

1. Golinska P, Wypij M, Agarkar G and Rathod D: Endophytic actinobacteria of medicinal plants: diversity and bioactivity. Ant Van Leeuw 2015; 108: 267–289.
2. Masand M, Jose PA, Menghani E and Jeba kumar SRD: Continuing hunt for endophytic actinomycetes as a source of novel biologically active metabolites. World. J. Microbiol. Biotechnol. 2015; 3: 1863–1875.
3. Zhang HW, Song YC and Tan RX: Biology and chemistry of endophytes. Zhang HW, Song YC, Tan RX: Biology and chemistry of endophytes. Nat. Prod. Res. 2006; 23: 753-771. DOI: 10.1039/b609472b 2006; 23: 753-771.
4. Rodriguez RJ and Redman R: More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. J. Exp Bot. 2008; 59: 1109-1114.
5. Kogel KH, Franken P and Huckelhoven R: Endophyte or parasite – what decides? Curr. Opin. Plant. Biol. 2006; 9: 358- 363.
6. Hendry SJ, Boddy L and Lonsdale D: Abiotic variables effect differential expression of latent infections in beech (*Fagus sylvatica*). New. Phytol. 2002; 155: 449-460.
7. Balandrin MF, Kloche JA, Wurtele ES and Bollinger WH: Natural plant chemicals: Sources of industrial and medicinal materials. Science 1985; 228: 1154-1160.
8. Zhang HR, Xiong YC, Zhao HY and Yi YJ: An antimicrobial compound from the endophytic fungus Phoma sp. isolated from the medicinal plant *Taraxacum mongolicum*. J. Taiwan Inst. Chem. Engrs. 2013; 44(2): 177-181.
9. Jalgaonwala R and Mahajan R: A review on microbial endophytes from plants: a treasure search for biologically active metabolites. Global Journal of Research on Medicinal Plants and Indigenous Medicine 2014; 3(6): 263–277.
10. Strobel G and Daisy B: Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev. 2003; 67: 491–502.
11. Taechowisan T, Lu C, Shen Y and Lumyong S: Secondary metabolites from endophytic *Streptomyces aureofaciens* CMUAc130 and their antifungal activity. Microbiology 2005; 151: 1691-1695.
12. Castillo U, Myers S, Browne L and Strobel G: Scanning electron microscopy of some *endophytic Streptomycetes* in Snakevine-Keennedia niggricans. Scanning 2006; 27: 305-311.
13. Igarashi Y, Trujillo ME, Martinez-Molina E and Yanase S: Antitumor anthraquinones from an endophytic actinomycetes *Micromonospora lupine* Sp. nov. Boorg Med Chem let. 2007; 17(13): 3702-3705.
14. Baritaux O, Richard H, Touche J and Derbesy M: Effects of drying and storage of herbs and spices on the essential oil. Part I. Basil, *Ocimum basilicum* L. Flavour Fragr. J. 1992; 27: 267–271.
15. Lawrence BM: A review of the world production of essential oils. Perfumer and Flavorist 1984; 10(5): 1-16.
16. Baytop T: Therapy with medicinal plants in Turkey (past and present). Istanbul University 1984.
17. Tilebeni HG: Review to basil medicinal plant. International Journal of Agronomy and Plant Production. 2011; 2(1): 5-7.
18. Waheeda K and Shyam KV: Formulation of Novel Surface Sterilization Method and Culture Media for the Isolation of Endophytic Actinomycetes from Medicinal Plants and its Antibacterial Activity. J Plant Pathol Microbiol 2017; 8: 2.
19. Good fellow M and Cross T: Classification in the biology of the actinomycetes (ed). Academic Press, London 1984; 7–164.
20. Thompson JD, Higgins DG, Gibson TJ and CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994; 24: 4673-4680.
21. Lei W, Peng Q, Xiu-Feng L and Shuai Z: Comparative analysis of chemical constituents, antimicrobial and

antioxidant activities of ethyl acetate extracts of *Polygonum cuspidatum* and its *endophytic actinomycete*, *Streptomyces* sp. As 0916. Chi J Nat Med. 2016; 14(2): 0117-0123.

22. Ismail M: Central properties and chemical composition of *Ocimum basilicum* essential oil. Pharm Biol 2006; 44(8): 619–626.
23. Sunaryanto R and Mahsunah AH: Isolation, purification and characterization of antimicrobial substances from endophytic actinomycetes. Makara J Sci 2013; 17-3.
24. Rao KVR and Rao TR: Molecular characterization and its antioxidant activity of a newly isolated Streptomyces coelicoflavus BC 01 from mangrove soil. J Young Pharm 2013; 5: 121-126.
25. Padmanabhan P and Jangle SN: Evaluation of *in vitro* anti-inflammatory activity of herbal preparation, a combination of four herbal plants. Int J Appl Basic Med Res. 2012; 2(1): 109-16.
26. Zampieri D, Mamolo MG, Laurini E and Scialino G: Antifungal and antimycobacterial activity of 1-(3, 5-diaryl-4, 5-dihydro-1H-pyrazol-4-yl)-1H-imidazole derivatives. Bioorg Med Chem. 2008; 16: 4516–4522.
27. McIntosh C, Demuth H, Pospisilik J and Pederson R: "Dipeptidyl peptidase IV inhibitors: How do they work as new antidiabetic agents. Regular Peptide 2005; 128(2): 159–65.
28. David LR: ed. CRC Handbook of Chemistry and Physics (90th ed.). Boca Raton, Florida: CRC Press, FL. 2009; 2804: 22.5_19cm. (Taylor and Francis Group). ISBN 978-1-4200-9084-0.
29. Fyaz MD, Ismail A, Dmitri O and Levitsky B: Aziridine alkaloids as potential therapeutic agents. Eur J Med Chem. 2009; 44: 3373–3387.
30. Sahu PK, Tomar AS, Waghmare S and Samant BS: Synthesis of Novel Pyrazole Amide Derivatives Containing a Pyridine Moiety. Chem Sci Rev let. 2014; 3(12): 987-989.
31. Khan KA, Hassan M, Faidallah M and Abdullah A: synthesis and biological evaluation of new N,N-Bis (1-substituted-ethylidene)-ethane1,2-diamine and their acetyl and trifluoroacetyl derivatives as cytotoxic and antimicrobial agents. J Chem. 2013; 1- 9.
32. Vasudevan A, Kalarickal V, Dileep P and Mandal K: Anti inflammatory property of n- Hexadecanoic acid: structural evidence and kinetic assessment. Chem Biol Drug Des. 2012; 80: 434–439.
33. Vijesh AM, Isloor AM, Telkar S and Peethambar SK: Synthesis, characterization and antimicrobial studies of some new pyrazole incorporated imidazole derivatives. Eur J Med Chem 2011; 46: 3531-3536.
34. Umesha KB, Rai KML and Harish Nayak MA: Antioxidant and antimicrobial activity of 5-methyl-2- (5-methyl-1, 3-diphenyl-1H-pyrazole-4-carbonyl) - 2, 4-dihydro-pyrazol-3-one. Int J Biomed Res. 2009; 5(4): 359-368.
35. Dahl S and Kaplan AM: 4-nitrophenyl ester, including mixed carbonates and bis carbonates, as leather fungicides. J Am Leather Chem Ass. 1960; 55: 480-500.
36. Pandey J, Tiwari VK, Verma SS and Chaturvedi V: Synthesis and antitubercular screening of imidazole derivatives. Eur J Med Chem. 2009; 44: 3350–3355.
37. Karnovsky MJ: A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J Cell Biol. 1965; 27: 137A 138A.
38. Fathy B, Wahab A, Khidre RE and Azolylimidazoles T: Synthetic strategies and medicinal applications. J Chem. 2014; 38: 1–27.
39. Valtcho D, Zheljazkov A and Callahan C: Central yield and oil composition of 38 basil (*Ocimum basilicum* L.) accessions grown in mississippi. J. Agric. Food Chem. 2008; 56: 241–245.
40. Trabanco AA, Aerts N, Alvarez RM and Andre´s IJ: 4-Phenyl-4-[1H-imidazol-2-yl]-piperidine derivatives as non-peptidic selective d-opioid agonists with potential anxiolytic / antidepressant properties. Part 2. Bioorg Med Chem lett. 2007; 17: 3860–3863.
41. Rosu T, Pahontu E, Ilies DC and Georgescu R: Synthesis and characterization of some new complexes of Cu(II), Ni(II) and V(IV) with Schiff base derived from indole-3-carboxaldehyde. Biological activity on prokaryotes and eukaryotes. Eur J Med Chem. 2012; 53: 380–389.
42. Smith MB and March J: Advanced organic chemistry: reactions, mechanisms, and structure, 5th ed. Wiley. Gerald Booth "Naphthalene Derivatives" in Ullmann's Encyclopedia of Industrial Chemistry 2005, Wiley-VCH, Weinheim.
43. Janmejai K, Srivastava E and Shankar SG: Chamomile: A herbal medicine of the past with bright future. NIH public acess 2011; 3(6): 895–901.
44. Banerjee M, Banerjee R and Kumar HKS: Medical significance of furan derivatives: Review. Int. J. Rev. Lif. Sci. 2012; 2(1): 7-16.
45. Aziz-ur-Rehman I, Ahmed MA, Abbasi K and Nafessa: Synthesis, Antibacterial and Lipoxygenase activities of N-[(Dimethyl substituted) phenyl]-N-(4-chlorophenyl)-4-chlorobenzenesulfonamides. Asian J Chem. 2015; 27: 1647-1650.
46. Foy BD and Smudde RA: William F. Wood. Preparation of 2-aminobenzaldehyde: A fragrant component of floral odors. J Chem Edu. 1993; 70(4): 322.
47. Soriano F, Zapardiel and Evanieto J: Antimicrobial susceptibilities of *Corynebacterium* species and other non-Spore-forming Gram-positive bacilli to 18 antimicrobial agents. Antimicrob. Agents Chemother. 1995; 39: 208–214.
48. Germaine K, Keogh E, Garcia-Cabellos G *et al*.: Colonization of poplar trees by gfp expressing bacterial endophytes. FEMS Microbiol Ecol. 2004; 48(1): 109-118.
49. Yu H, Zhang L, Li L, Zheng C, Guo L, Li W, Sun P and Qin L: Recent developments and future prospects of antimicrobial metabolites produced by endophytes. Microbiol Res. 2010; 165(6): 437–449.
50. Li J, Zhao GZ, Huang HY and Qin S: Isolation and characterization of culturable endophytic actinobacteria associated with *Artemisia annua* L. Ant Van Leeuw 2012; 101(3): 515–527.
51. Sheng Q, Xing K, Ji-Hong Jiang and Li-Hua X: Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. Appl Microbiol Biotechnol. 2011; 89: 457–473.
52. Narendhran S, Rajiv P, Vanathi P and Rajeshwari S: Spectroscopic analysis of bioactive compounds from *Streptomyces cavouresis* kuv39: evaluation of antioxidant and cytotoxicity activity. Int J Pharm Pharm Sci. 2014; 6 (7): 319-322.
53. Pandey AK, Singh P and Tripathi NN: Chemistry and bioactivities of essential oils of some *Ocimum* species: an overview. Asian Pac J Trop Biomed. 2014; 4(9): 682-694.
54. Wani MC, Taylor HL, Wall ME, Coggon P and Mc Phail AT: The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J Am Chem Soc. 1971; 93: 2325–7.

55. Gunther E: Ethnobotany of western Washington University of Washington Publications in Anthropology 1945; 10: 1-62.
56. Stierle A, Strobel G, Stierle D and Grothaus P: The search for a taxol-producing microorganism among the endophytic fungi of the Pacific yew, *Taxus brevifolia*. J Nat Prod. 1995; 58: 1315–24.
57. Ezra D, Castillo UF, Strobel GA and Hess WM: Coronamycins, peptide antibiotics produced by a verticillate *Streptomyces* sp. (MSU- 2110) endophytic on *Monstera* sp. Microbiol. 2004: 150(4): 785-793.
58. Puri SC, Verma V, Amna T, Qazi GN, and Spiteller MJ: An endophytic fungus from *Nothapodytes foetida* that produces camptothecin. J Nat Prod. 2005; 68: 1717-1719.
59. Atsuko M and Yōko T: Endophytic actinomycetes: promising source of novel bioactive compounds. J. Antibiot 2017; 1–6.
60. Tanvir R, Sajid I and Hasnain S: Biotechnological potential of endophytic actinomycetes associated with Asteraceae plants: isolation, biodiversity and bioactivities. Biotechnol Lett. 2014; (36)4: 767–773.
61. Liu X, Dong MX, Chen M and Jiang XL: Antioxidant activity and phenolics of an endophytic *Xylaria* sp. from *Ginkgo biloba*. Food Chem. 2007; 105(2): 548–554.
62. Silva GH, Teles LH, Zanardi LM and Young MC: Cadinane sesquiterpenoids of *Phomopsis cassiae* an endophytic fungus associated with *Cassia spectabilis* (Leguminosae). Phytochemistry 2007; 67: 1969

How to cite this article:
Khanam W and Vootla SK: Comparative study of *Ocimum basillicum* and its *Endophytic actinomycetes Streptomyces flavoviridis* A3WK: evaluation of antioxidant, anti-inflammatory and antimicrobial activity. Int J Pharm Sci & Res 2018; 9(3):1023-34. doi: 10.13040/IJPSR. 0975-8232.9(3).1023-34.