2nd International Electronic Conference on Medicinal Chemistry

1-30 November 2016
chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

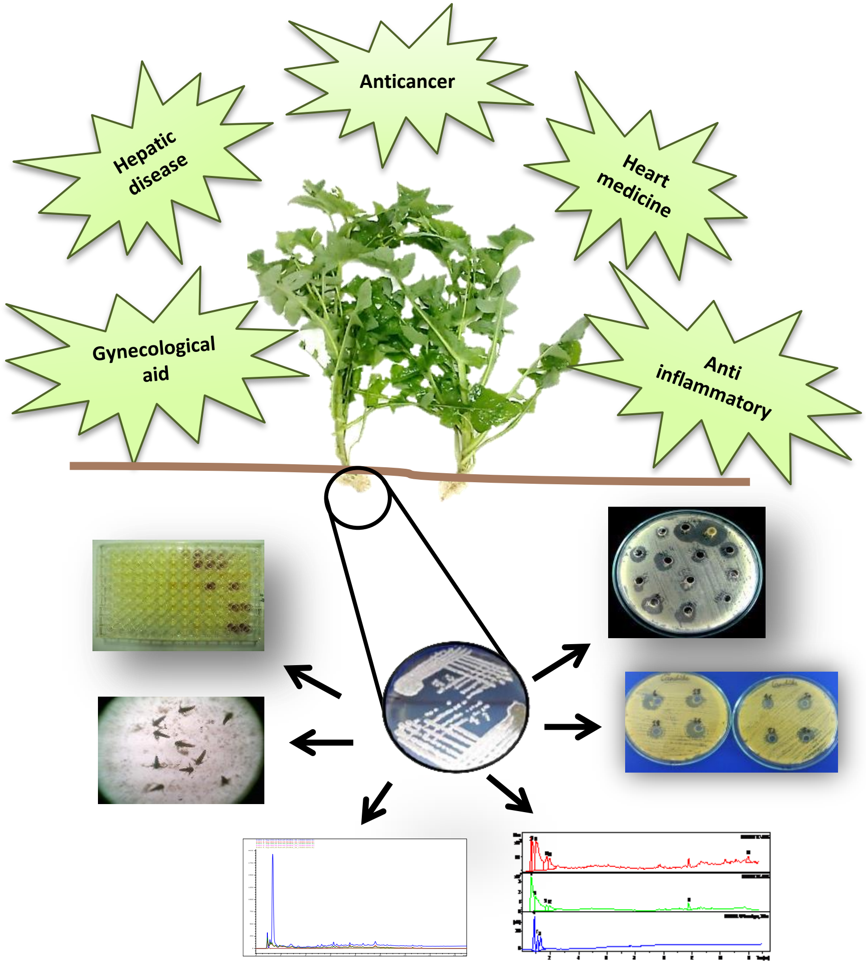
# Bioprospecting of Asteraceae medicinal plants of Pakistan for their associated bioactive endophytic actinomycetes for new drug targets

**Rabia Tanvir[1,2,3]*, Imran Sajid[2], Shahida Hasnain[2,4], Andreas Kulik[5] and Stephanie Grond[3]**

[1]University Diagnostic Lab (UDL), University of Veterinary and Animal Sciences (UVAS), 54000, Lahore, Punjab Pakistan;
[2]Department of Microbiology and Molecular Genetics, University of the Punjab, Quaid-e-Azam Campus, 54590, Lahore, Punjab Pakistan;
[3]Institut für Organische Chemie, Auf Der Morgenstelle 18A, Eberhard Karls Universität Tübingen, 72076, Tübingen, Germany;
[4]Department of Microbiology and Molecular Genetics, The Women University, Multan Punjab Pakistan;
[5]Mikrobiologie/Biotechnologie**,** Interfakultäres Institut für Mikrobiologie und Infektionsmedizin, Eberhard Karls Universität Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany
***** Corresponding author: rabia.tanvir@uvas.edu.pk

# Bioprospecting of Asteraceae medicinal plants of Pakistan for their associated bioactive endophytic actinomycetes for new drug targets

## Graphical Abstract



**Abstract:**
Since the beginning of mankind, plants have been used as the source of medicinal agents thereby becoming a major course to discovering new drugs. The practice of using traditional medicine is prevalent in Pakistan that has a rich history of herbal plants being used by Hakims in folk medicine (Unani medicine). The Asteraceae family is the largest plant family in Pakistan, with plants of considerable medicinal importance. Endophytes include all organisms that symptomless colonize the living internal tissues of their hosts during a variable period of their lifetime. There they produce a broad variety of bioactive secondary metabolites with unique structure that are advantageous for the plant. Endophytic actinomycetes also colonize the internal tissues of plants without causing any visible changes or damage. They exploit an unusual habitat and considering this, this may enable them to possess the potential to produce bioactive compounds as similar to their host plant. Our study explores the bioprospecting potential related to endophytic actinomycetes of Asteraceae medicinal plants of Pakistan. After isolation and identification the endophytes were screened for their bioactive metabolites potential for new drug targets. This included extensive biological and chemical screening. The bioactive compounds were purified through column chromatography and final identification was done through HPLC-MS and NMR. The purified compounds were observed to be extremely potent with promising antimicrobial potential against major pathogens including algae and fungal strains as well as possessing antioxidant and cytotoxic potential.

**Keywords:** Actinomycetes; Asteraceae; Endophytes; Medicinal plants; Pakistan

# Introduction

- Natural products
    - Compounds acquired from natural sources
        - Such as from plants, animals and microorganisms [1]

- Medicinal plants as sources of new natural products
    - Such products are
        - Easily accessibility having less side effects, toxicity and better biodegradability
        - Cheaper, safer and environmentally friendly [2]
        - Structurally unique active compounds
        - Potential for new drug discovery [3]

[1] Baker, D. D., M. Chu, U. Oza, and V. Rajgarhia. "The value of natural products to future pharmaceutical discovery." *Nat Prod Rep* 24, no. 6 (2007): 1225-44.
[2] Soković, M. D., Glamočlija, J. M., and Ćirić, A. D. "Natural products from plants and fungi as fungicides." In *Fungicides-Showcases of Integrated Plant Disease Management from Around the World*; Nita, M., Ed.; In Tech: 2003; p 185-232.
[3] Berdy, J. "Bioactive microbial metabolites." *Journal of Antibiotics* 58, no. 1 (2005): 1-26.

# Introduction (Cont.)

- Pakistan
  - Rich in medicinal herbs
    - Traditionally used to prepare herbal medicines by Hakims (Traditional physicians)
    - Used for centuries in the Unani system of medicine (folk medicine)
    - About 80% of the population is dependent on it
      - Particularly in villages and rural areas [4]

- *Asteraceae* plant family
- Dicotyledonous flowering plants
- Largest plant family in Pakistan
  - Over 650 species distributed in 15 tribes [5]

[4] Qureshi, Rizwana Aleem, Mushtaq Ahmed, and M Asad Ghufran. "Indigenous knowledge of some important wild plants as a folk medicines in the area of Chhachh (Distt. Attock) Punjab, Pakistan." *EJEAFChe* 6, no. 11 (2007): 2500-2511.
[5] Hussain, Javid, Zia Muhammad, Riaz Ullah, Farman Ullah Khan, I Ullah, Naeem Khan, Javed Ali, and Saleem Jan. "Evaluation of the chemical composition of *Sonchus eruca* and *Sonchus asper*." *J. Am. Sci* 6, no. 9 (2010): 231-35.

# Introduction (Cont.)

- *Ageratum conyzoides* L.
  - Neeli booti [6]
- Folk medicines
  - Ulcers, pneumonia, diarrhea
  - Burn wounds
  - Leprosy, gynecological diseases [7]
- Anti larvicidal activity
  - *Culex quinquefasciatus*
  - *Aedes aegypti*
  - *Anopheles stephensi* [8]
- Phytochemical screening
  - Alkaloids, resins, saponins, tannins, glycosides and flavonoids [9]

*A.conyzoides* growing opposite the department of Microbiology and Molecular Genetics, University of the Punjab, Lahore Pakistan. Photo courtesy: Dr. Rabia Tanvir


[6] Zafar, Muhammad, MA Khan, Mushtaq Ahmad, and Shazia Sultana. "Palynological and taxonomic studies of some weeds from flora of Rawalpindi." Paper presented at the Pakistan Journal of Weed Science Research 12, (2006): 99-109.
[7] Okunade, Adewole L. "*Ageratum conyzoides* L. (Asteraceae)." *Fitoterapia* 73, no. 1 (2002): 1-16.
[8] Ming, Lin Chau. "*Ageratum conyzoides*: A tropical source of medicinal and agricultural products." *Perspectives on new crops and new uses* (1999): 469-73.
[9] Nasrin, Fatema. "Antioxidant and cytotoxic activities of *Ageratum conyzoides* stems." *International Current Pharmaceutical Journal* 2, no. 2 (2013): 33-37.

# Introduction (Cont.)

- *Sonchus oleraceus* L.
  - Local name, 'Dodak'

- Folk medicines
  - Anticancer agent, anti diarrheal
  - Anti inflammatory, blood purifier
  - Gynecological aid, heart medicine
  - Hepatic diseases, vermicide [10]
  - Rich in
    - Antioxidants
    - Polyphenols
    - vitamin C
    - Four times more antioxidant activity than blueberry extracts [11]

*S.oleraceus* growing next to the GeneLab in the department of Microbiology and Molecular Genetics, University of the Punjab, Lahore Pakistan. Photo courtesy: Dr. Rabia Tanvir

[10] Jimoh, Florence O, Adeolu A Adedapo, and Anthony J Afolayan. "Comparison of the nutritive value, antioxidant and antibacterial activities of *Sonchus asper* and *Sonchus oleraceus*." *Records of Natural Products* 5, (2011): 29-42.
[11] Ou, Zong-Quan, David M. Schmierer, Thomas Rades, Lesley Larsen, and Arlene McDowell. "Application of an online post-column derivatization HPLC-DPPH assay to detect compounds responsible for antioxidant activity in *Sonchus oleraceus* leaf extracts." *Journal of Pharmacy and Pharmacology* 65, no. 2 (2013): 271-79.

# Introduction (Cont.)

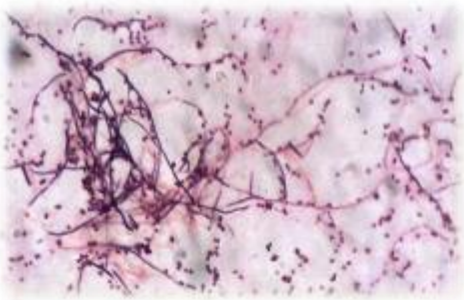
- Endophytes
  - Microorganisms that reside intra and/or intercellular in the tissues of plants
  - Do not cause any symptoms
  - Produce bioactive secondary metabolites with unique structure
  - Antibiotics, immunosuppressants, antioxidants, and anticancer agents [12]

- Advantages to the plant
  - Fitness enhancements
    - Increased resistance to herbivores
    - Parasitism
    - Drought
    - Growth enhancements [13]

[12] Pimentel, Mariana Recco, Gustavo Molina, Ana Paula Dionísio, Mário Roberto Maróstica Junior, and Gláucia Maria Pastore. "The use of endophytes to obtain bioactive compounds and their application in biotransformation process." *Biotechnology Research International* 2011: 2-11.
[13] Firáková, Silvia, Mária Šturdíková, and Marta Múčková. "Bioactive secondary metabolites produced by microorganisms associated with plants." *Biologia* 62, no. 3 (2007): 251-257.

# Introduction (Cont.)

- Endophytic actinomycetes
  - Gram positive filamentous bacteria
  - Reside within the plants
  - Exploit an unusual habitat
  - Novel bioactive compounds [14]



Observation of endophytic actinomycete as Gram positive filamentous rods. Photo courtesy: Dr. Rabia Tanvir


Purified endophytic actinomycete strains on GYM agar plates. Photo courtesy: Dr. Rabia Tanvir

[14] Rungin, S., C. Indananda, P. Suttiviriya, W. Kruasuwan, R. Jaemsaeng, and A. Thamchaipenet. "Plant growth enhancing effects by a siderophore-producing endophytic Streptomycete isolated from a Thai jasmine rice plant (*Oryza sativa* L. cv. KDML105)." *Antonie Van Leeuwenhoek* 102, no. 3 (2012): 463-72.

# Results and discussion

- 145 endophytic actinomycetes were isolated
  - *Ageratum conyzoides* (AG) 52
  - *Sonchus oleraceus* (SO) 93

- Frequency of isolation
  - Roots RS = 139
  - Shoots SS= 2
  - Leaves LS = 4

| Plant sample | Plant parts | Isolates |
|---|---|---|
| *Ageratum conyzoides* | Cut roots | AGRS 1, AGRS 2, AGRS 3, AGRS 4, AGRS 5, AGRS 6, AGRS 7, AGRS 8, AGRS 9, AGRS 10, AGRS 11, AGRS 12, AGRS 13, AGRS 14, AGRS 15, AGRS 16, AGRS 17, AGRS 18, AGRS 19, AGRS 20, AGRS 21, AGRS 22, AGRS 23, AGRS 24, AGRS 29, AGRS 33, AGRS 34, AGRS 35, AGRS 36, AGRS 37, AGRS 38, AGRS 39, AGRS 40, AGRS 41, AGRS 42, AGRS 43, AGRS 44, AGRS 45, AGRS 46, AGRS 47, AGRS 48, AGRS 49, AGRS 50, AGRS 51, AGRS 52, AGRS 53 |
| | Cut shoots and leaves | AGL 1, AGLS 1, AGLS 2, AGLS 3, AGSS 1, AGSS 2 |
| *Sonchus oleraceus* | Cut roots | SORS 1, SORS 2, SORS 3, SORS 4, SORS 20, SORS 21, SORS 22, SORS 23, SORS 24, SORS 25, SORS 26, SORS 28, SORS 32, SORS 33, SORS 34, SORS 35, SORS 37, SORS 41, SORS 42, SORS 43a, SORS 43b, SORS 44, SORS 46, SORS 48, SORS 49, SORS 50, SORS 51, SORS 52, SORS 56, SORS 59, SORS 61, SORS 62, SORS 64a, SORS 64b, SORS 66, SORS 67, SORS 68, SORS 70, SORS 71, SORS 73, SORS 74, SORS 76, SORS 77, SORS 79, SORS 80, SORS 82, SORS 83, SORS 84, SORS 85, SORS 86, SORS 87, SORS 88, SORS 89, SORS 90, SORS 91, SORS 92, SORS 93, SORS 95, SORS 96, SORS 97, SORS 98, SORS 99, SORS 100, SORS 101, SORS 102, SORS 103, SORS 104, SORS 105, SORS 106, SORS 107, SORS 108, SORS 109, SORS 112, SORS 113, SORS 114, SORS 115, SORS 116, SORS 117, SORS 118, SORS 119, SORS 120, SORS 122, SORS 126, SORS 127, SORS 130, SORS 131, SORS 136, SORS 140, SORS 144, SORS 146, SORS 151, SORS 157, SORS 173 |
| | Cut shoots and leaves | None |

# Results and discussion (Cont.)

- Preliminary antimicrobial activity test

- GYM broth [15] with actinomycetes growth tested
- Prominently bioactive against *Bacillus subtilis*, *Staphlococcus aureus*, *Escherichia coli*, *Pseudomonas* and *Enterobacter* sp.

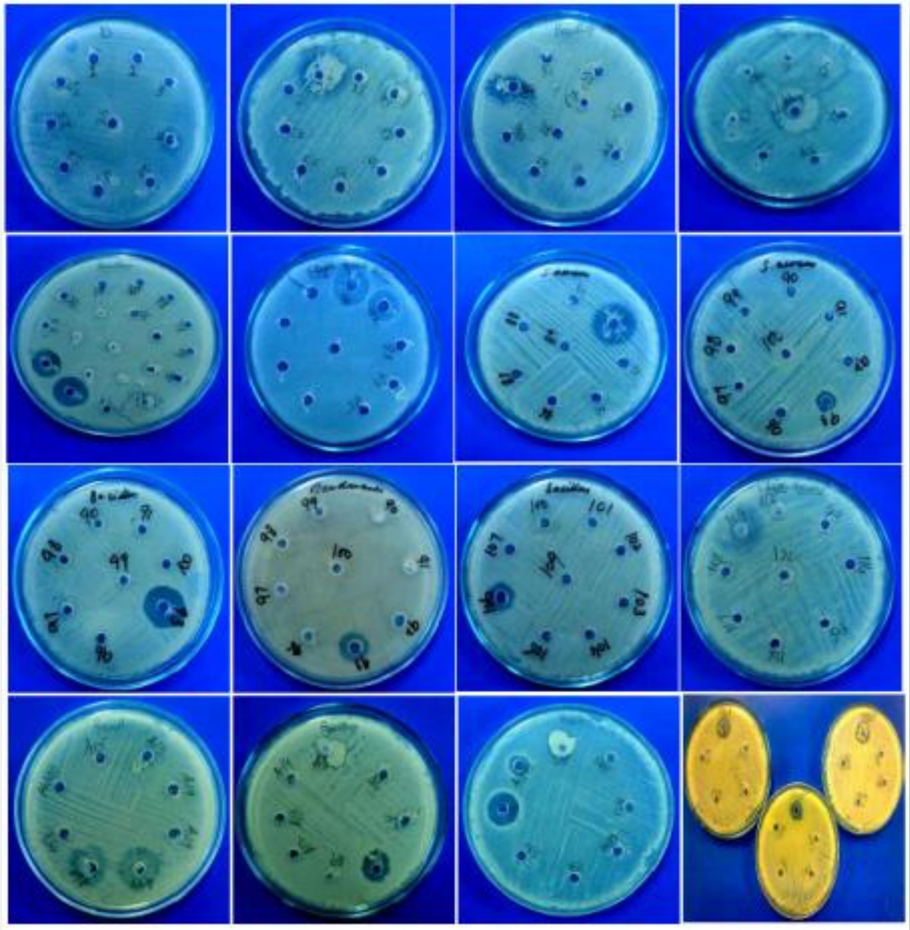


Photo courtesy: Dr. Rabia Tanvir

[15] Shirling, E. T. and Gottlieb, D. "Method for Characterization of Streptomyces Species." Int. J. Syst, 16, (1966): 313-340.

# Results and discussion (Cont.)

- Screening of endophytic actinomycetes
  - Selected endophytic actinomycetes with most preliminary bioactivity
  - Crude extraction
    - Sonication and 1:1 ethyl acetate [16]
    - Biological and chemical screening

- Biological screening

1. Antimicrobial activity testing
    - Agar well diffusion method [17]
      - ATCC standard organisms
      - Multi drug resistant pathogens
      - Clinically isolated fungi
      - Microalgae

[16] Tanvir, Rabia, Imran Sajid, Shahida Hasnain, Andreas Kulik, and Stephanie Grond. "Rare actinomycetes *Nocardia caishijiensis* and *Pseudonocardia carboxydivorans* as endophytes, their bioactivity and metabolites evaluation." *Microbiological Research* 185 (2016): 22-35.
[17] Gebreyohannes, Gebreselema, Feleke Moges, Samuel Sahile, and Nagappan Raja. "Isolation and characterization of potential antibiotic producing actinomycetes from water and sediments of Lake Tana, Ethiopia." *Asian Pacific Journal of Tropical Biomedicine* 3, no. 6 (2013): 426-35.

# Results and discussion (Cont.)

| Strain no. | Zone of Inhibition (mm) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Test Organisms | | | | | | | | | | |
| | *B. subtilis ATCC 6051* | *Ent. spp.* | *Pseudo . spp.* | *S. aureus ATCC 25923* | *E. coli ATCC 25922* | *K. pneumonia ATCC 706003* | *MRSA* | *E. coli K12* | *S. cerevisiae ATCC 9080* | *C. tropicalis* | *C. vulgaris* |
| AGRS 1 | 17 | 14 | – | 11 | 12 | 10 | 5 | 7 | – | 20 | – |
| AGRS 14 | 13 | 15 | 11 | – | 15 | – | 15 | – | – | 20 | 14 |
| AGRS 16 | 10 | – | – | – | – | – | – | – | – | 20 | 10 |
| AGRS 19 | 14 | 12 | 13 | 3 | 12 | 13 | 10 | – | – | – | 13 |
| AGRS 44 | 9 | 10 | 7 | 9 | 14 | 12 | 10 | – | 10 | 10 | 10 |
| AGRS 49 | 9 | 10 | 14 | 12 | 14 | 13 | 12 | 6 | – | 20 | – |
| AGSS 1 | – | – | – | – | – | – | – | – | – | – | 12 |
| SORS 4 | 14 | 13 | 10 | 10 | 15 | 10 | – | – | – | 25 | 13 |
| SORS 26 | 9 | 11 | 7 | 10 | 10 | – | 8 | – | – | 15 | – |
| SORS 32 | 10 | 10 | 10 | 11 | 15 | – | – | – | – | 20 | 10 |
| SORS 45 | 14 | 12 | 17 | 14 | 14 | 14 | 12 | – | – | 14 | 10 |
| SORS 82 | 15 | 11 | 15 | 14 | 14 | 15 | 10 | – | – | 14 | – |
| SORS 95 | 10 | 10 | 11 | – | 15 | 13 | 10 | – | – | 17 | 10 |
| SORS 106 | 15 | 14 | 14 | 5 | 14 | 13 | 11 | – | – | 17 | 10 |
| SORS 119 | 12 | 12 | 12 | 11 | 10 | 15 | 11 | – | – | 20 | 10 |
| SORS 124 | 15 | 10 | – | 7 | – | 7 | – | – | – | 20 | 10 |
| SORS 146 | 10 | 8 | – | 7 | – | 7 | – | – | – | 20 | 10 |

Key: *B.subtilis Bacillus subtilis; Ent.spp., Enterobacter spp.; Pseudo spp., Pseudomonas spp.; S. aureus Staphlococcus aureus; E.coli Escherichia coli; K. pneumoniae Klebsiella pneumoniae; MRSA Methicillin Resistant Staphlococcus aureus; S. cerevisiae, Saccharomyces cerevisiae; C. tropicalis Candida tropicalis, C.vulgaris Chlorella vulgaris*

(-) no inhibition

60µl crude extract was added in each well

Values are means of triplicates studies

# Results and discussion (Cont.)

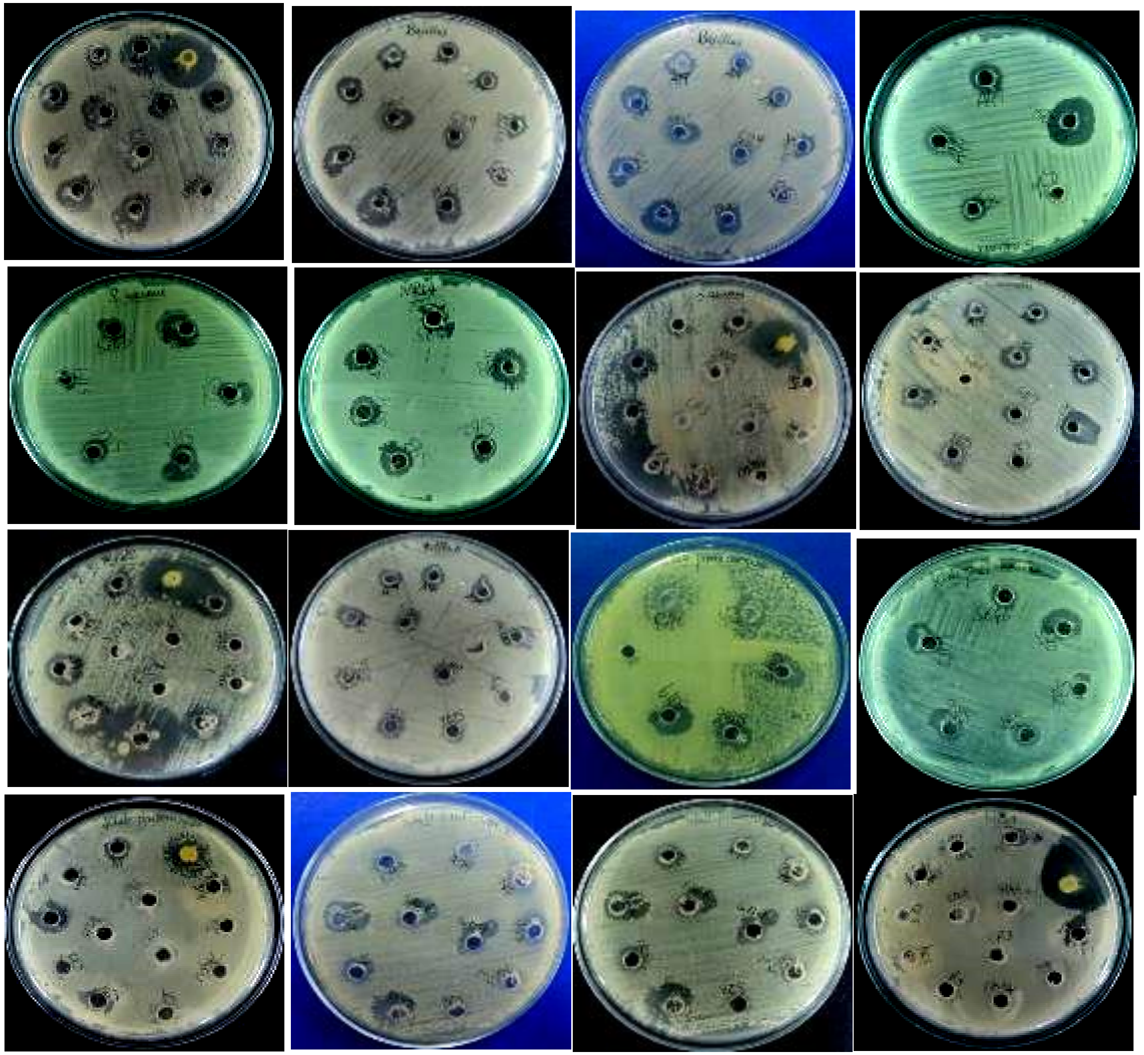


Photo courtesy: Dr. Rabia Tanvir

# Results and discussion (Cont.)

## 2. Brine shrimp cytotoxicity assay [18]

- Crude extract
- *Artemia salina* nauplii
- 10, 50, 100, 150, 500, 1000μg/ml
  - Increase in cytotoxicity with increased concentration

[18] Tanvir, Rabia, Imran Sajid, and Shahida Hasnain. "Biotechnological potential of endophytic actinomycetes associated with Asteraceae plants: Isolation, biodiversity and bioactivities." *Biotechnology Letters* 36, no. 4 (2014): 767-73.

# Results and discussion (Cont.)

| Strain no. | % mortality under the concentration studied (μg/mL$^{-1}$) | | | | | | LC$_{50}$ (μg/mL$^{-1}$) | 95% confidence interval | |
|---|---|---|---|---|---|---|---|---|---|
| | 10 | 50 | 100 | 150 | 500 | 1000 | | Lower bound | Upper bound |
| DMSO (1% in seawater) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| AGRS1 | 10.4 | 23.3 | 28.0 | 52 | 63.3 | 68.5 | 500 | 3.718394 | 47.12732 |
| AGRS14 | 33.7 | 34.7 | 59.3 | 62.1 | 63.2 | 69.9 | 360 | 9.560784 | 60.33922 |
| AGRS44 | 54.1 | 56.6 | 60.6 | 70 | 74 | 76 | 100 | 16.7756 | 74.9282 |
| AGRS49 | 36.6 | 51.6 | 59.9 | 62.5 | 63.3 | 82.8 | 242 | 12.73336 | 63.52993 |
| SORS4 | 21.8 | 22.2 | 52.3 | 56.4 | 60.6 | 63.3 | 500 | 3.713851 | 51.83552 |
| SORS26 | 39 | 53.3 | 60 | 63.3 | 65.5 | 68.9 | 300 | 13.14654 | 64.19523 |
| SORS32 | 34.9 | 55.3 | 58.6 | 60.6 | 68.9 | 100 | 200 | 12.21982 | 61.2479 |
| SORS45 | 29 | 37.9 | 42.9 | 43.6 | 57.1 | 58.6 | 600 | 9.704513 | 47.8246 |
| SORS82 | 32.1 | 33.3 | 36.6 | 37.9 | 43.3 | 45 | 1000 | 9.931168 | 43.25934 |
| SORS95 | 41.3 | 53.5 | 56 | 62.9 | 72 | 80.7 | 220 | 14.02904 | 65.28805 |
| SORS106 | 34.1 | 44 | 44.9 | 46.5 | 60 | 72.4 | 400 | 11.33607 | 50.32279 |
| SORS119 | 39 | 43 | 47.2 | 66.6 | 75 | 100 | 200 | 11.38434 | 57.29794 |
| SORS124 | 29.5 | 37.4 | 38.5 | 46 | 50 | 65.5 | 580 | 9.73186 | 44.75168 |
| SORS146 | 41 | 42.3 | 47.7 | 47.9 | 57.1 | 92.5 | 300 | 11.20848 | 50.63012 |

Values are means of triplicate studies, $n = 15$ (number of nauplii used). Score for LC$_{50}$: Highly toxic - <20(μg/mL$^{-1}$), toxic – upto 1000 μg/mL$^{-1}$ and non toxic - >1000μg/mL$^{-1}$, LC$_{50}$ was calculated through Probit

# Results and discussion (Cont.)

**Disintegration of body parts observed**

Photo courtesy: Dr. Rabia Tanvir

# Results and discussion (Cont.)

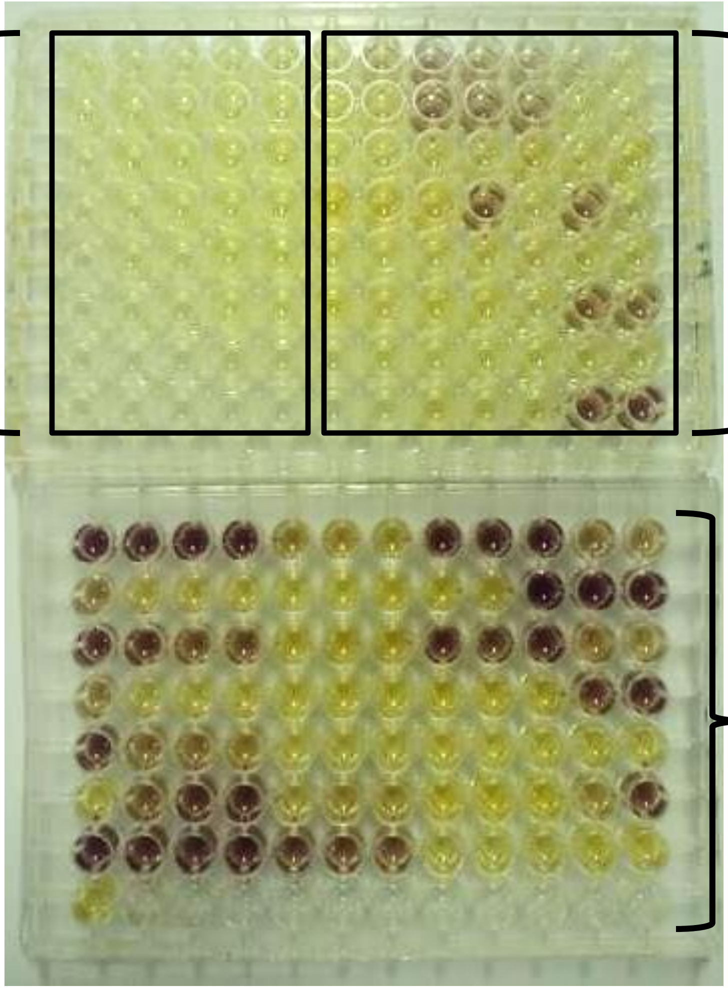
## 3. Antioxidant activity test

– Using the 2, 2'-diphenyl-1-picrylhydrazyl free radical (DPPH) assay [18]

| Strain no. | DPPH Scavenging activity (%) under the concentration studied ($\mu g/mL^{-1}$) | | | | | $EC_{50}$ ($\mu g/mL^{-1}$) |
|---|---|---|---|---|---|---|
| | 100 | 500 | 1000 | 5000 | 10000 | |
| Ascorbate standard | 100 | 100 | 100 | 100 | 100 | 0.338 |
| AGRS1 | 4.2 | 23.5 | 42.7 | 62 | 81.2 | 0.904 |
| AGRS8 | 16.3 | 16.7 | 33.9 | 65.3 | 92.4 | 0.962 |
| AGRS14 | 11 | 42.6 | 49.7 | 55.4 | 95.8 | 0.879 |
| AGRS44 | 23.5 | 55.4 | 59.5 | 60.4 | 100 | 0.820 |
| SORS4 | 14.2 | 40 | 50.6 | 65.3 | 91.1 | 0.879 |
| SORS26 | 25 | 29.2 | 34.1 | 42.4 | 85 | 0.922 |
| SORS32 | 25 | 33.7 | 36.9 | 79 | 90.1 | 0.908 |
| SORS45 | 7.5 | 13 | 22.5 | 34.6 | 50.6 | 0.944 |
| SORS82 | 6.2 | 32 | 38.6 | 42.2 | 62.5 | 0.828 |
| SORS106 | 38.8 | 40 | 50.7 | 75 | 92.5 | 0.859 |
| SORS119 | 8.7 | 10 | 28.4 | 37.7 | 40.5 | 0.830 |
| SORS146 | 27.5 | 28.3 | 29.7 | 42.2 | 95 | 0.935 |

Values are means of triplicate studies

# Results and discussion (Cont.)



Ascorbic acid standard

Endophytic actinomycetes crude extract

Endophytic actinomycetes crude extract

Photo courtesy: Dr. Rabia Tanvir

# Results and discussion (Cont.)

## 4. Embryotoxicity test

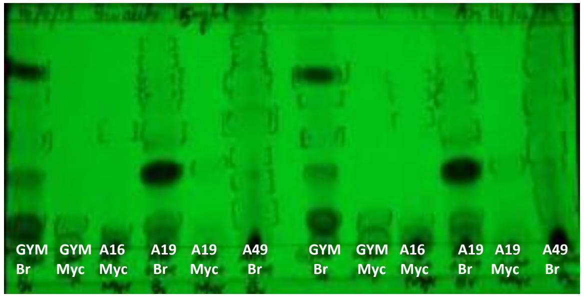
- 'Desi breed' chicken embryo
- Inoculated with extracts
- incubated at 37˚C for 7 days

| Strain no. | Embryotoxicity against chicken embryo (%) under the concentration studied (μg/mL$^{-1}$) | |
|---|---|---|
| | 100 | 500 |
| Control | Normal embryo formation | Normal embryo formation |
| AGRS8 | No embryo formation. Coagulated protein, blackened with $H_2S$ gas production | No embryo formation. Solid egg yolk with coagulated proteins, blackened with $H_2S$ gas production |
| AGRS19 | No embryo formation. Coagulated protein, blackened with $H_2S$ gas production | No embryo formation. Coagulated protein, blackened with $H_2S$ gas production |
| AGRS49 | No embryo formation. Coagulated protein, blackened with $H_2S$ gas production | No embryo formation. Coagulated protein, blackened with more production of $H_2S$ gas |
| SORS32 | No embryo formation. Coagulated protein, blackened with $H_2S$ gas production | No embryo formation. Coagulated protein, blackened with $H_2S$ gas production |
| SORS106 | No embryo formation. Coagulated protein, blackened and greenish with $H_2S$ gas production | No embryo formation. Coagulated protein, blackened and greenish with $H_2S$ gas production |
| SORS119 | No embryo formation. Coagulated protein, blackened and greenish | No embryo formation. Coagulated protein, blackened and greenish |

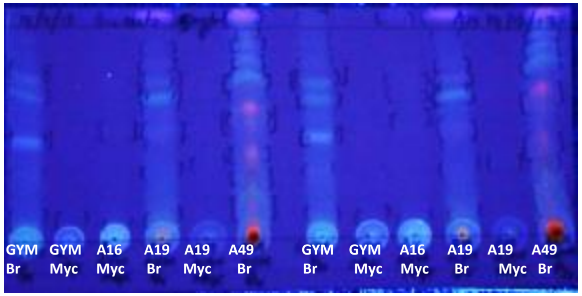
Five eggs were used for each strain; ten un-inoculated eggs were used as control

# Results and discussion (Cont.)

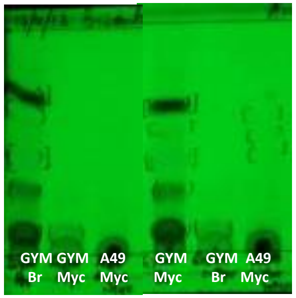
- Chemical screening
  - Thin Layer Chromatography (TLC)
  - 365 nm and 254nm
  - Diverse metabolite production



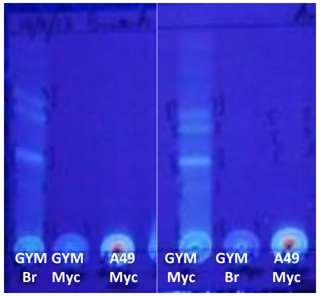

**(a) 254nm**




**(b) 365nm**




**(c) 254nm**




**(d) 365nm**

**Photo courtesy: Dr. Rabia Tanvir**

# Results and discussion (Cont.)

- Bioautography

- Polar, medium polar and non polar bands
- Yellow area indicated antimicrobial activity against *E.coli*

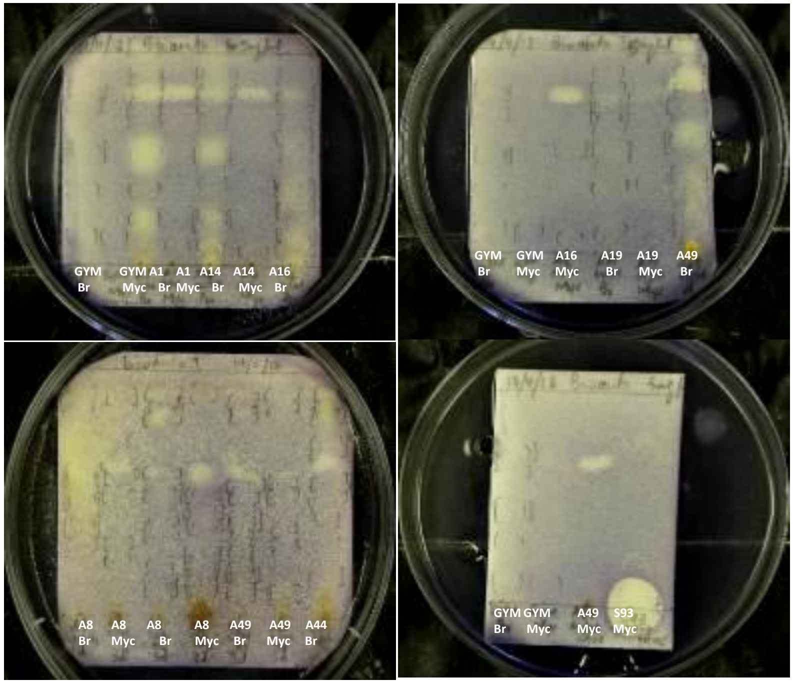



Photo courtesy: Dr. Rabia Tanvir

# Results and discussion (Cont.)

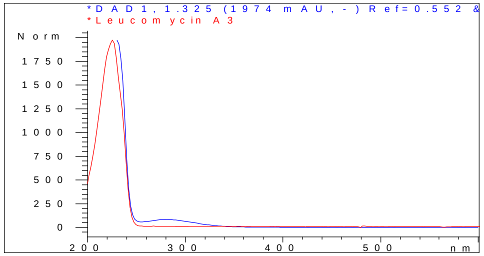
| Isolates | No. of nucleotides sequenced (bp) | % Similarity with | | Gen Bank accession numbers |
|---|---|---|---|---|
| AGRS 8 | 1238 | **Streptomyces albovinaceus* | 99% | KC191695 |
| SORS45 | 666 | *Streptomyces diastatochromogenes* | 99% | KC191698 |
| SORS106 | 1166 | **Streptomyces badius* | 99% | KC191702 |

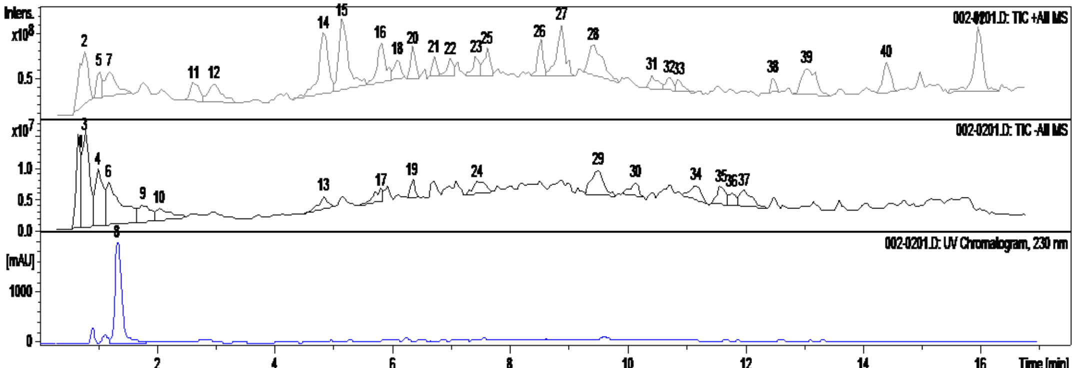
*Tanvir, Rabia, Imran Sajid, and Shahida Hasnain. "Biotechnological potential of endophytic actinomycetes associated with Asteraceae plants: Isolation, biodiversity and bioactivities." *Biotechnology Letters* 36, no. 4 (2014): 767-73.

*Tanvir, Rabia, Imran Sajid, and Shahida Hasnain. "Larvicidal potential of Asteraceae family endophytic actinomycetes against *Culex quinquefasciatus* mosquito larvae." *Natural Product Research* 28, no. 22 (2014): 2048-52.

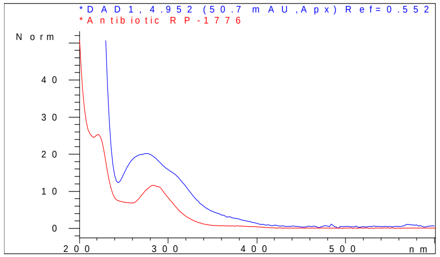
# Results and discussion (Cont.)

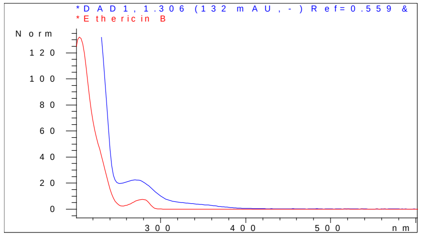
- High Performance Liquid Chromatography Mass Spectrometry Diode Array Detector (HPLC-MS-DAD)
  - DAD-UV vis database search in the natural substance library [19]
  - UV ranges of 230, 260, 280, 360 and 435nm
  - A peak similarity observed between compounds in the database



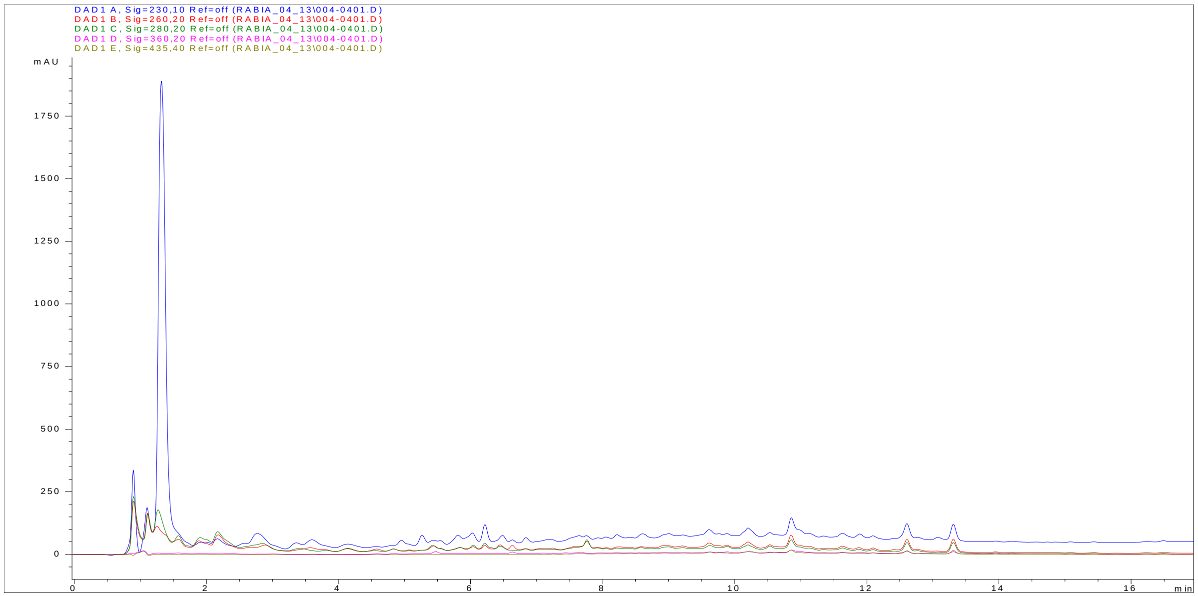

Total Ion Chromatogram of AGRS 8 extract with UV absorption spectra at 230nm.



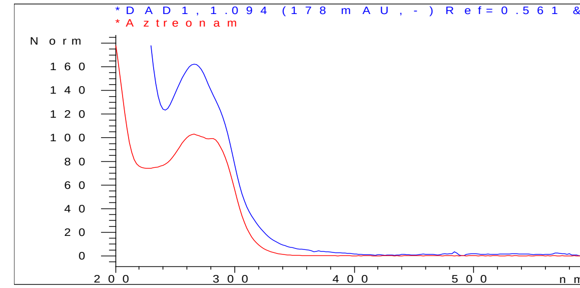



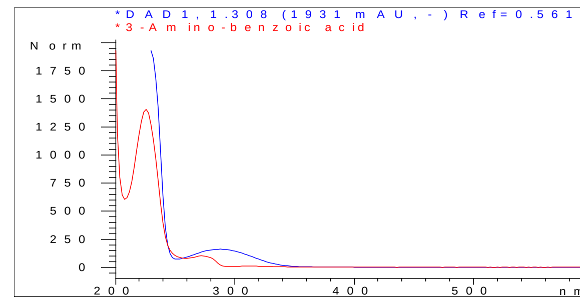

[19] Fiedler, Hans-Peter. "Biosynthetic capacities of actinomycetes. 1 Screening for secondary metabolites by HPLC and UV-visible absorbance spectral libraries." *Natural Product Letters* 2, no. 2 (1993): 119-28.

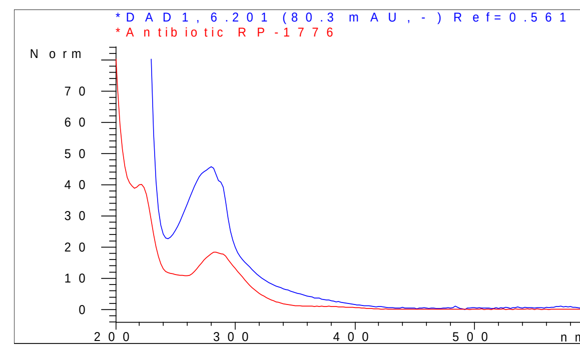
# Results and discussion (Cont.)




Total UV absorption spectra of AGRS 44 extract with UV absorption spectra at 230, 260, 280, 360, 435nm



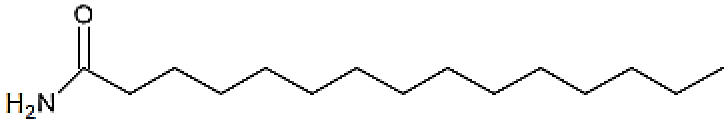



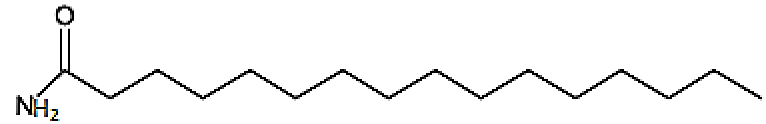
# Results and discussion (Cont.)

- $^{1}H$ NMR (600 MHz)
  - Structures were drawn using the software ChemDraw®
  - Comparison of the identified compound with the reference compound
  - Long chained fatty acid amide derivatives
    - Widely bioactive

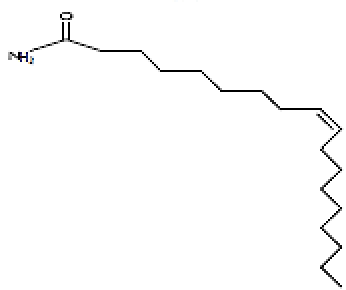
# Results and discussion (Cont.)



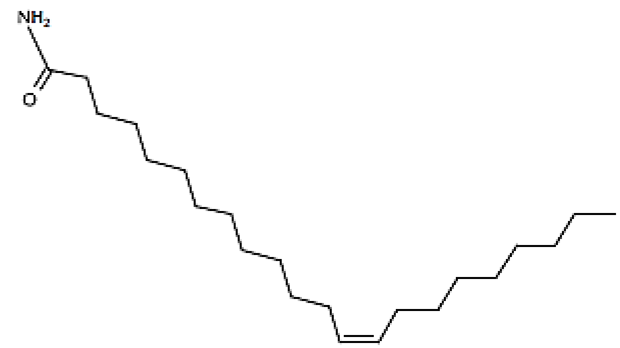

**Tetradecanamide**




**N-Hexadecanamide**



**9- Octadecenamide**




**13- Docosenamide**

## Conclusions

- *Asteraceae* plants used in the study
  - Medically important in a wide variety of aliments
  - Evidence of the production of the active metabolites by the endophytic actinomycetes residing within these plants

- Endophytes of *Asteraceae* plant family
  - Diversity of endophytic actinomycetes isolated from different plant parts
  - Biological screening revealed
    - Wide spectrum of biotechnologically significant compounds
      - Antimicrobial, cytotoxic, antioxidant and embryotoxic agents
      - Dose dependent response

# Conclusions (Cont.)

- Chemical screening revealed
  - Producing non polar, medium polar and polar bioactive compounds
- HPLC-MS-DAD
  - Interesting matches but with slight precision
- $^{1}H$ NMR
  - Fatty acid amides and derivatives
  - None reported from endophytes
  - Reported as anti tumor, anti infective, cardiovascular, nervous system agent, anti-inflammatory, immune agent, and enzyme inhibitor [20]
- Further exploration of this novel ecological niche
  - Novel metabolites
  - Biotechnological industries

[20] Ledroit, Véronique, Cécile Debitus, Catherine Lavaud, and Georges Massiot. "Jaspines a and B: Two New Cytotoxic Sphingosine Derivatives from the Marine Sponge Jaspis Sp." *Tetrahedron letters* 44, no. 2 (2003): 225-28.

## Acknowledgments


- The Higher Education Commission (HEC) Pakistan is greatly acknowledged for the grant 1-/HEC/HRD/2012/2301 under the IRSIP program to the recipient Dr. Rabia Tanvir

- Dr. Yi Zhang at the Guangdong Ocean University China provided the *E. coli* K-12 strain for which the authors are greatly thankful