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# Detection of Bioactive Chemical Compounds of *Achillea millefolia* and *Glycine max* Using FTIR spectroscopic profile and Evaluation of Its Anti-microbial Activity

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***ABSTRACT***

***The aims of this study were analysis of the secondary metabolite products of Achillea millefolia and Glycine max and evaluation of Anti-fungal activity. The FTIR analysis of Achillea millefolia proved the presence of functional group assignment Alkenes, Alkyl halides and Alkane with Intensity 779.24 Bending (Strong =C–H), 1018.41 Stretch (Strong C-F), 1074.35 Stretch (Strong C-F), 1153.43 Stretch (Strong C-F), 1313.52 Stretch (Strong C-F), 2927.94 Stretch (Strong C-H) The FTIR analysis of Glycine max proved the presence of functional group assignment Alkyl halides and Alkane with Intensity 1016.1 Stretch (Strong C-F), 1029.2 Stretch (Strong C-F), 1238.1 Stretch (Strong C-F), 1373.7 Stretch (Strong C-F), 2920.4 Stretch (Strong C-H). Zone of inhibition (mm) of test bacterial strains to Achillea millefolia bioactive compounds and standard antibiotics were (4.970±0.32), (3.115±0.13), (4.871±0.15), (4.766±0.31), and (3.990±0.22) uses Achillea millefolia bioactive compounds, and (1.001±0.09), (1.771±0.41), (1.008±0.12), (0.009±0.01), and (2.001±0.13) uses Rifambin, and (1.008±0.11), (2.682±0.29), (2.860±0.13), (1.037±0.21), and (1.000±0.10) uses Streptomycin, and (0.730±0.12), (1.000±0.46), (0.330±0.10), (2.000±0.11), and (0.084±0.10) uses Kanamycin, and (1.104±0.26), (1.996±0.27), (1.009±0.10), (1.005±0.15), and (1.007±0.11) uses Cefotoxime for Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas eurogenosa respectively. Zone of inhibition (mm) of test bacterial strains to Glycine max bioactive compounds and standard antibiotics were (3.007±0.21), (4.006±0.14), (5.000±0.30), (3.089±0.21), and (4.000±0.23) uses Glycine max bioactive compounds, and (2.905±0.10), (2.9001±0.12), (2.006±0.12), (1.972±0.10), and (3.719±0.14) uses Rifambin, and (2.127±0.10), (1.000±0.11), (1.991±0.10), (2.994±0.11), and (0.988±0.10) uses Streptomycin, and (1.069±0.09), (2.961±0.10), (1.094±0.10), (3.190±0.11), and (1.009±0.10) uses Kanamycin and (2.000±0.11), (1.000±0.11), (2.371±0.11), (0.077±0.10), and (2.113±0.12) uses Cefotoxime for Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas eurogenosa respectively.***

***Keywords: Achillea millefolia, Glycine max, FTIR, Anti-microbial Activity***

## 1. INTRODUCTION

A metabolite is an intermediate or end product of metabolism.[1] The term metabolite is usually used for small molecules. Metabolites have various functions, including fuel, structure, signaling, stimulatory and inhibitory effects on enzymes and interactions with other organisms [2]. A primary metabolite is directly involved in normal "growth", development, and reproduction. Ethylene exemplifies a primary metabolite produced large-scale by industrial microbiology. A secondary metabolite is not directly involved in those

processes, but usually has an important ecological function. Examples include antibiotics and pigments such as resins and terpenes etc. A primary metabolite is a kind of metabolite that is directly involved in normal growth, development, and reproduction. It usually performs a physiological function in the organism (i.e. an intrinsic function). A primary metabolite is typically present in many organisms or cells [3]. It is also referred to as a central metabolite, which has an even more restricted meaning (present in any autonomously growing cell or organism). Some common examples of primary metabolites include: lactic acid, and certain amino acids. Note that primary metabolites do not show any pharmacological actions or effects. Secondary metabolites also called Specialized Metabolites [4], secondary products or Natural Products are organic compounds produced by bacteria, fungi, or plants which are not directly involved in the normal growth, development, or reproduction of the organism. Unlike primary metabolites, absence of secondary metabolites does not result in immediate death, but rather in a long-term impairment of the organism's survivability, fecundity [5], or aesthetics, or perhaps in no significant change at all. Specific secondary metabolites are often restricted to a narrow set of species within a phylogenetic group. Secondary metabolites often play an important role in plant defense against herbivory and other interspecies defenses. Humans use secondary metabolites as medicines, flavourings, pigments, and recreational drugs [6]. Secondary metabolites aid a host in important functions such as protection, competition, and species interactions, but are not necessary for survival. One important defining quality of secondary metabolites is their specificity. Usually, secondary metabolites are specific to an individual species,[7-10] though there is considerable evidence that horizontal transfer across species or genera of entire pathways plays an important role in bacterial (and, likely, fungal) evolution.[11-15] Research also shows that secondary metabolism can affect different species in varying ways. In the same forest, four separate species of arboreal marsupial folivores reacted differently to a secondary metabolite in eucalypts.[16-19] This shows that differing types of secondary metabolites can be the split between two herbivore ecological niches. Additionally, certain species evolve to resist secondary metabolites and even use them for their own benefit. For example, monarch butterflies have evolved to be able to eat milkweed (Asclepias) despite the toxic secondary metabolite it contains. This ability additionally allows the butterfly and caterpillar to be toxic to other predators due to the high concentration of secondary metabolites consumed. Fourier-transform infrared spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range [20]. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time. The goal of absorption spectroscopy techniques (FTIR, ultraviolet-visible ("UV-Vis") spectroscopy, etc.) is to measure how much light a sample absorbs at each wavelength [21-23]. The most straightforward way to do this, the "dispersive spectroscopy" technique, is to shine a monochromatic light beam at a sample, measure how much of the light is absorbed, and repeat for each different wavelength. (This is how some UV–vis spectrometers work, for example.)

## 2. Materials and Methods

### Extraction and purification of the antibacterial agent:

Antibacterial compounds were recovered from *Achillea millefolia* and *Glycine max* by solvent extraction with methanol in a ratio of 1:1 (v/v) and shaken well for 1 h. The methanol phase was separated and evaporated to dryness in water bath at 80 - 90°C. Residue was weighed and redissolved with little methanol [24].

**Preparation of sample**

About 20 grams of the plant sample powdered were soaked in 100 ml methanol for 16 hours in a rotatory shaker. Whatman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis. It was again filtered through sodium sulphate in order to remove the traces of moisture.

**Fourier transform infrared spectrophotometer (FTIR)**

The powdered sample of *Achillea millefolia and Glycine max* was treated for FTIR spectroscopy (Shimadzu, IR Affinity, Japan). The sample was run at infrared region between 400 nm and 4000 nm [25, 26].

**Determination of antimicrobial activity of crude bioactive compounds of *Achillea millefolia and Glycine max***

The test pathogens were swabbed in Müller-Hinton agar plates. Sixty $\mu L$ of plant extract was loaded on the bored wells. Antibacterial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control. The antibacterial activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

## 3. RESULTS AND DISCUSSION

**Identification of biochemical compounds**

The FTIR analysis of *Achillea millefolia* proved the presence of functional group assignment Alkenes, Alkyl halides, Amide, and Alkane with Intensity 819.75 Bending (Strong), 1018.41 Stretch (Strong), 1238.30 Stretch (Strong), 1379.10 Stretch (Strong), 1614.42 Stretch (Bending), 2850.79 Stretch (Strong), 2920.23Stretch (Strong). The FTIR analysis of *Glycine max* proved the presence of functional group assignment Alkenes, Alkyl halides, Amide, Acid and Alkane with Intensity 875.68 Bending (Strong =C–H), 1016.49 Stretch (Strong C-F), 1024.20 Stretch (Strong C-F), 1197.79 Stretch (Strong C-F), 1317.38 Stretch (Bending N-H), 1716.65 Stretch (Strong C=O), 2850.79 Stretch (Strong C-H), 2922.16 Stretch (Strong C-H). Fourier-transform spectroscopy is a less intuitive way to obtain the same information. Rather than shining a monochromatic beam of light (a beam composed of only a single wavelength) at the sample, this technique shines a beam containing many frequencies of light at once and measures how much of that beam is absorbed by the sample. This process is rapidly repeated many times over a short time span. Afterwards, a computer takes all this data and works backward to infer what the absorption is at each wavelength. The beam described above is generated by starting with a broadband light source—one containing the full spectrum of wavelengths to be measured. The light shines into a Michelson interferometer—a certain configuration of mirrors, one of which is moved by a motor. As this mirror moves, each wavelength of light in the beam is periodically blocked, transmitted, blocked, transmitted, by the interferometer, due to wave interference. Different wavelengths are modulated at different rates, so that at each moment the beam coming out of the interferometer has a different spectrum. As mentioned, computer processing is required to turn the raw data (light absorption for each mirror position) into the desired result (light absorption for each wavelength). The processing required turns out to be a common algorithm called the Fourier transform. The Fourier transform converts one domain (in this case displacement of the mirror in cm) into its inverse domain (wavenumbers in cm−1). The raw data is called an "interferogram". Infrared spectroscopy provides a useful method for herbal analysis and elucidate the compounds structures as well as for quantitative analysis of drugs. Recently, a number of plants have been reported for antibacterial properties across the world. It is hoped that this study would direct to the establishment of some compounds that could be used to invent new and more potent antibacterial drugs of natural origin. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy

and bioactivity. Zone of inhibition (mm) of test bacterial strains to *Achillea millefolia* bioactive compounds and standard antibiotics were (4.970±0.32), (3.115±0.13), (4.871±0.15), (4.766±0.31), and (3.990±0.22) uses *Achillea millefolia* bioactive compounds, and (1.001±0.09), (1.771±0.41), (1.008±0.12), (0.009±0.01), and (2.001±0.13) uses Rifambin, and (1.008±0.11), (2.682±0.29), (2.860±0.13), (1.037±0.21), and (1.000±0.10) uses Streptomycin, and (0.730±0.12), (1.000±0.46), (0.330±0.10), (2.000±0.11), and (0.084±0.10) uses Kanamycin, and (1.104±0.26), (1.996±0.27), (1.009±0.10), (1.005±0.15), and (1.007±0.11) uses Cefotoxime for *Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas eurogenosa* respectively. Zone of inhibition (mm) of test bacterial strains to *Glycine max* bioactive compounds and standard antibiotics were (3.007±0.21), (4.006±0.14), (5.000±0.30), (3.089±0.21), and (4.000±0.23) uses *Glycine max* bioactive compounds, and (2.905±0.10), (2.9001±0.12), (2.006±0.12), (1.972±0.10), and (3.719±0.14) uses Rifambin, and (2.127±0.10), (1.000±0.11), (1.991±0.10), (2.994±0.11), and (0.988±0.10) uses Streptomycin, and (1.069±0.09), (2.961±0.10), (1.094±0.10), (3.190±0.11), and (1.009±0.10) uses Kanamycin and (2.000±0.11), (1.000±0.11), (2.371±0.11), (0.077±0.10), and (2.113±0.12) uses Cefotoxime for *Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas eurogenosa* respectively. The differences in the susceptibilities of Gram positive and Gram negative bacteria to *Streptomyces* extracts have been observed by previous workers . Gram negative bacteria are inherently more resistant to antimicrobials than Gram positive organisms and this has been ascribed to the combined exclusion of antimicrobial compounds by double membrane barrier and transmembrance efflux present in this group of organisms.

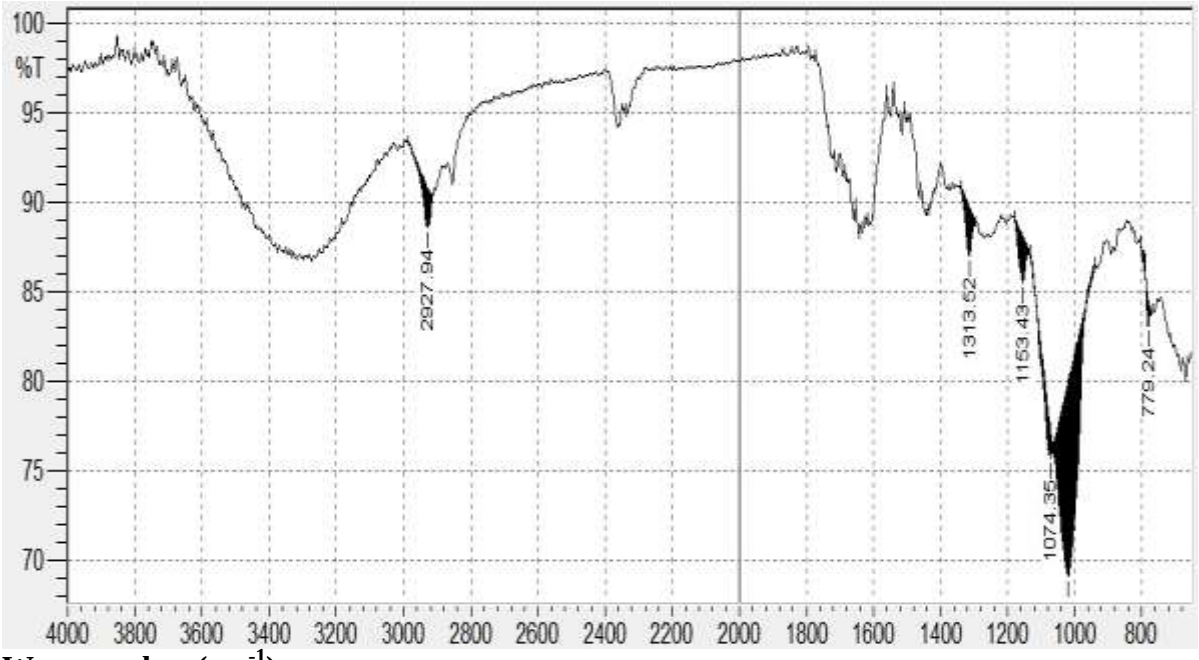


**Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Achillea millefolia***

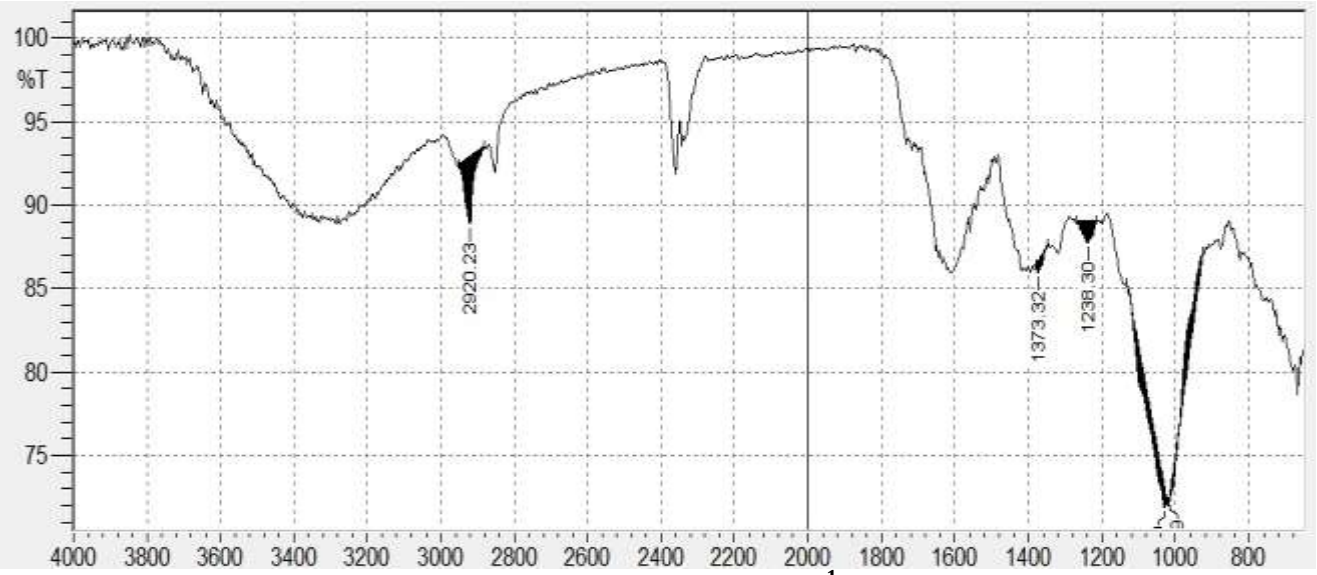


**Figure 2. Fourier-transform infrared spectroscopic profile solid analysis of *Glycine max***

**Table 1. FT-IR peak values of solid analysis of *Achillea millefolia*.**

| No. | Peak (Wave number cm-ˡ) | Intensity | Corr. Intensity | Type of Intensity | Bond | Type of Vibration | Functional group assignment | Group frequency |
|---|---|---|---|---|---|---|---|---|
| **1.** | 779.24 | 83.137 | 1.335 | Strong | =C–H | Bending | Alkenes | 650-1000 |
| **2.** | 1018.41 | 69.123 | 10.890 | Strong | C-F | Stretch | alkyl halides | 1000-1400 |
| **3.** | 1074.35 | 75.727 | 1.869 | Strong | C-F | Stretch | alkyl halides | 1000-1400 |
| **4.** | 1153.43 | 85.542 | 2.542 | Strong | C-F | Stretch | alkyl halides | 1000-1400 |
| **5.** | 1313.52 | 87.060 | 2.666 | Strong | C-F | Stretch | alkyl halides | 1000-1400 |
| **6.** | 2927.94 | 88.603 | 2.172 | Strong | C-H | Stretch | Alkane | 2850-3000 |

**Table 2. FT-IR peak values of solid analysis of *Glycine max*.**

| No. | Peak (Wave number cm-ˡ) | Intensity | Corr. Intensity | Type of Intensity | Bond | Type of Vibration | Functional group assignment | Group frequency |
|---|---|---|---|---|---|---|---|---|
| **1.** | 1016.1 | 72.194 | 0.312 | Strong | C-F | Stretch | alkyl halides | 1000-1400 |
| **2.** | 1029.2 | 71.928 | 1.185 | Strong | C-F | Stretch | alkyl halides | 1000-1400 |
| **3.** | 1238.1 | 87.765 | 1.308 | Strong | C-F | Stretch | alkyl halides | 1000-1400 |
| **4.** | 1373.7 | 85.935 | 0.753 | Strong | C-F | Stretch | alkyl halides | 1000-1400 |
| **5.** | 2920.4 | 88.891 | 4.040 | Strong | C-H | Stretch | Alkane | 2850-3000 |

**Table 3. Zone of inhibition (mm) of test bacterial strains to *Achillea millefolia* bioactive compounds and standard antibiotics.**

| / *Achillea millefolia* Antibiotics | Bacteria | | | | |
|---|---|---|---|---|---|
| | *Staphylococcus aureus* | *Escherichia Coli* | *Proteus Mirabilis* | *Klebsiella Pneumonia* | *Pseudomonas eurogenosa* |
| *Achillea millefolia* | 4.970±0.32 | 3.115±0.13 | 4.871±0.15 | 4.766±0.31 | 3.990±0.22 |
| Rifambin | 1.001±0.09 | 1.771±0.41 | 1.008±0.12 | 0.009±0.01 | 2.001±0.13 |
| Streptomycin | 1.008±0.11 | 2.682±0.29 | 2.860±0.13 | 1.037±0.21 | 1.000±0.10 |

| | | | | | |
|---|---|---|---|---|---|
| Kanamycin | 0.730±0.12 | 1.000±0.46 | 0.330±0.10 | 2.000±0.11 | 0.084±0.10 |
| Cefotoxime | 1.104±0.26 | 1.996±0.27 | 1.009±0.10 | 1.005±0.15 | 1.007±0.11 |

**Table 4. Zone of inhibition (mm) of test bacterial strains to *Glycine max* bioactive compounds and standard antibiotics.**

| / *Glycine max* Antibiotics | Bacteria | | | | |
|---|---|---|---|---|---|
| | *Staphylococcus aureus* | *Escherichia Coli* | *Proteus Mirabilis* | *Klebsiella Pneumonia* | *Pseudomonas eurogenosa* |
| *Glycine max* | 3.007±0.21 | 4.006±0.14 | 5.000±0.30 | 3.089±0.21 | 4.000±0.23 |
| Rifambin | 2.905±0.10 | 2.9001±0.12 | 2.006±0.12 | 1.972±0.10 | 3.719±0.14 |
| Streptomycin | 2.127±0.10 | 1.000±0.11 | 1.991±0.10 | 2.994±0.11 | 0.988±0.10 |
| Kanamycin | 1.069±0.09 | 2.961±0.10 | 1.094±0.10 | 3.190±0.11 | 1.009±0.10 |
| Cefotoxime | 2.000±0.11 | 1.000±0.11 | 2.371±0.11 | 0.077±0.10 | 2.113±0.12 |

## 4. CONCLUSION

The FTIR analysis of *Achillea millefolia* proved the presence of functional group assignment Alkenes, Alkyl halides. The FTIR analysis of *Glycine max* proved the presence of functional group assignment Alkyl halides and Alkane. Zone of inhibition (mm) of test bacterial strains to *Achillea millefolia* bioactive compounds and standard antibiotics were (4.970±0.32), (3.115±0.13), (4.871±0.15), (4.766±0.31), and (3.990±0.22) uses *Achillea millefolia* bioactive compounds for *Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas eurogenosa* respectively. Zone of inhibition (mm) of test bacterial strains to *Glycine max* bioactive compounds and standard antibiotics were (3.007±0.21), (4.006±0.14), (5.000±0.30), (3.089±0.21), and (4.000±0.23) uses *Glycine max* bioactive compounds for *Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas eurogenosa* respectively.

## 5. REFERENCES

1. Aghcheh, R, Kubicek, C. P. Epigenetics as an emerging tool for improvement of fungal strains used in biotechnology. Appl Microbiol Biotechnol. 2015; 99(15): 6167–6181
2. Marmann, A., Aly, A. H., Lin, W., Wang, B., Proksch, P. Co-Cultivation – A Powerful Emerging Tool for Enhancing the Chemical Diversity of Microorganisms. Mar Drugs. 2014; 12: 1043–1065
3. Netzker, T. Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. Front Microbiol 2015; 6: 299.
4. Bertrand, S. et al. Metabolite induction via microorganism co-culture: A potential way to enhance chemical diversity for drug discovery. Biotechnol Adv. 2014; 32(6): 1180–1204
5. Frisvad, J. C, Samson, R. A. Polyphasic taxonomy of Penicillium subgenus Penicillium A guide to identifcation of food and airborne terverticillate Penicillia and their mycotoxins. Stud Mycol. 2004; 49: 1–174.
6. Kanetis, L., Förster, H, Adaskaveg, J. E. Determination of natural resistance frequencies in Penicillium digitatum using a new airsampling method and characterization of Fludioxonil- and Pyrimethanil-Resistant isolates. Phytopathology. 2010; 100, 738–743
7. Strano, M. C., Altieri, G., Admane, N., Genovese, F., Di Renzo, G. C. Advance in Citrus Postharvest Management: Diseases, Cold Storage and Quality Evaluation. In: Gill, H. & Garg, H. Citrus pathology. 2017; 7,139–159

8. Charousova, I., J. Medo, E. Halenarova and S. Javorekova,. Antimicrobial and enzymatic activity of actinomycetes isolated from soils of coastal islands. J. Adv. Pharm. Technol. Res. 2017; 8: 46-51.
9. Cho, K.H., S.T. Kim and Y.K. Kim, 2007. Purification of a pore-forming peptide toxin, tolaasin, produced by *Pseudomonas tolaasii* 6264. BMB Rep., 40: 113-118.
10. Coleman, J.J., S. Ghosh, I. Okoli and E. Mylonakis, 2011. Antifungal activity of microbial secondary metabolites. Plos One, Vol. 6. 10.1371/journal.pone.0025321
11. Demain, A.L. and S. Sanchez. Microbial drug discovery: 80 years of progress. J. Antibiot., 62: 5-16.
12. Demain, A.L. Pharmaceutically active secondary metabolites of microorganisms. Applied Microbiol. Biotechnol., 1999; 52: 455-463.
13. Desai, D.A., G.P. Kukreja, C.J. Raorane and S.B. Patil, 2016. Partial Sequencing of Serendipitously Isolated Antifungal Producer, Pseudomonas tolaasii Strain GD 76 16s Ribosomal RNA Gene. Int. J. Curr. Microbiol. Applied Sci., 2009; 5: 455-458.
14. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. Evolution. 1985; 39: 783-791.
15. Singh A, Mehta S, Singh HB, Nautiyal CH. Biocontrol of collar rot disease of betelvine (Piper betle L.) caused by Sclerotium rolfsii by using rhizosphere-competent Pseudomonas fluorescens NBRI-N6 and P. fluorescens NBRI-N. Curr Microbiol. 2003; 47: 153-8.
16. El-Tarabily KA, Soliman MH, Nassar AH, Al-Hassani HA, Sivasithamparam K, McKenna F, St J Hardy GE. Biological control of Sclerotinia minor using a chitinolytic bacterium and actinomycetes. Plant Pathol. 2000; 49: 573-83.
17. Gohlke, Roland S.; McLafferty, Fred W. "Early gas chromatography/mass spectrometry". Journal of the American Society for Mass Spectrometry. 1993; 4 (5): 367–371.
18. Hites, Ronald A. "Development of Gas Chromatographic Mass Spectrometry". Analytical Chemistry. 2016; 88 (14): 6955–6961.
19. Brock, David C. A Measure of Success". Chemical Heritage Magazine. 2011; 29 (1).
20. Wang, T.; Lenahan, R. "Determination of volatile halocarbons in water by purge-closed loop gas chromatography". Bulletin of Environmental Contamination and Toxicology. 1984; 32 (1): 429–438.
21. Tsivou, M.; Kioukia-Fougia, N.; Lyris, E.; Aggelis, Y.; Fragkaki, A.; Kiousi, X.; Simitsek, P.; Dimopoulou, H.; Leontiou, I. -P.; Stamou, M.; Spyridaki, M. -H.; Georgakopoulos, C. An overview of the doping control analysis during the Olympic Games of 2004 in Athens, Greece". Analytica Chimica Acta. 2006; 555: 1–13.
22. Smith, P. A.; Lepage, C. J.; Lukacs, M.; Martin, N.; Shufutinsky, A.; Savage, P. B. "Field-portable gas chromatography with transmission quadrupole and cylindrical ion trap mass spectrometric detection: Chromatographic retention index data and ion/molecule interactions for chemical warfare agent identification". International Journal of Mass Spectrometry. 2010; 295 (3): 113–118.
23. Sloan, K. M.; Mustacich, R. V.; Eckenrode, B. "Development and evaluation of a low thermal mass gas chromatograph for rapid forensic GC-MS analyses". Field Analytical Chemistry & Technology. 2001; 5 (6): 288–301.
24. Brault, James W. New Approach to high-precision Fourier transform spectrometer design". Applied Optics. 1996; 35 (16): 2891–2896.
25. Connes, J.; Connes, P. "Near-Infrared Planetary Spectra by Fourier Spectroscopy. I. Instruments and Results". Journal of the Optical Society of America. 1966; 56(7): 896–910.

26. Smith, D.R.; Morgan, R.L.; Loewenstein, E.V. "Comparison of the Radiance of Far-Infrared Sources". J. Opt. Soc. Am. 1968; 58 (3): 433–434.