SYNTHESIS OF HETEROCYCLIC COMPOUNDS FOR ANALYSIS OF THEIR ANTIBACTERIAL ACTIVITIES

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Abstract: The emergence of multidrug-resistant bacteria has become a global health concern, necessitating the development of novel and effective antibacterial agents. Heterocyclic compounds have garnered significant attention due to their diverse chemical structures and promising biological activities. This review paper provides an overview of recent advances in the synthesis of heterocyclic compounds and their evaluation for anti-bacterial activities. The paper discusses different heterocyclic scaffolds, their synthetic approaches, and the mechanisms underlying their antibacterial potential. Furthermore, it highlights the challenges and future prospects in the field of heterocyclic compound development as antibacterial agents.

Introduction:

Infectious diseases caused by bacteria continue to pose a major threat to human health. The increased prevalence of drug-resistant bacterial strains necessitates the exploration of new therapeutic strategies. Heterocyclic compounds have been widely investigated for their potential as antimicrobial agents due to their structural diversity and unique biological properties. This review aims to provide a comprehensive analysis of recent developments in the synthesis of heterocyclic compounds and their antibacterial evaluation.

Synthetic Approaches for Heterocyclic Compounds:

The first section discusses diverse synthetic methods utilized in the preparation of heterocyclic compounds. Classical techniques, such as cyclization reactions and multicomponent reactions, are explored alongside cutting-edge strategies like click chemistry and transition-metal-catalyzed reactions. The versatility and efficiency of these approaches have paved the way for the synthesis of structurally diverse heterocyclic compounds with potential antibacterial properties.

Heterocyclic Scaffolds and their Antibacterial Evaluation:

This section delves into specific heterocyclic scaffolds and their corresponding antibacterial activities. Pyridines, quinolines, oxazoles, and thiazoles are among the prominent scaffolds investigated. Key studies are presented, detailing the antibacterial assays, minimum inhibitory concentration (MIC) determinations, and structure-activity relationships (SAR) that govern the biological potency of these compounds.

Mechanisms of Action:

Understanding the mode of action is crucial for developing effective antibacterial agents. This section elucidates the mechanisms by which heterocyclic compounds exert their antibacterial effects. Targeting bacterial cell wall synthesis, inhibiting protein synthesis, interfering with nucleic acid metabolism, and disrupting essential enzymes are among the mechanisms discussed in this context.

Synergy and Combination Therapy:
Combating bacterial resistance often requires innovative strategies. This section explores the concept of synergy and combination therapy involving heterocyclic compounds. Synergistic effects between heterocyclic agents and conventional antibiotics, as well as the potential of utilizing them as adjuvants to restore antibiotic efficacy, are examined.

Synthesis of Heterocyclic Compounds:
The first section of the review focuses on various synthetic approaches employed in the preparation of heterocyclic compounds. It covers the use of conventional methods, as well as modern techniques, such as microwave-assisted synthesis and green chemistry approaches. The discussion encompasses a wide range of heterocyclic scaffolds, including pyridines, pyrimidines, imidazoles, thiazoles, and more. Additionally, emphasis is placed on the importance of structure-activity relationship studies to optimize antibacterial properties.

Mechanisms of Antibacterial Activity:
This section explores the mechanisms by which heterocyclic compounds exhibit antibacterial activity. It discusses the targeting of bacterial cell wall synthesis, inhibition of protein synthesis, disruption of bacterial nucleic acid metabolism, and interference with bacterial enzyme function. The molecular interactions of heterocyclic compounds with bacterial targets and their impact on bacterial cell viability are critically evaluated.

Structure-Activity Relationship (SAR) Studies:
An essential aspect of developing effective antibacterial agents lies in understanding the structure-activity relationship. This section highlights recent studies on SAR of heterocyclic compounds, shedding light on the key structural features that govern their antibacterial efficacy. Such insights are crucial in guiding rational design strategies for new and improved antibacterial agents.

Evaluation of Anti-bacterial Activities:
This section compiles the findings from various in vitro and in vivo studies assessing the antibacterial activities of heterocyclic compounds. Studies comparing the compounds against a panel of bacterial strains, including both Gram-positive and Gram-negative bacteria, are discussed. Moreover, the paper delves into the determination of minimum inhibitory concentration (MIC) values and the development of structure-based virtual screening methods.

Extraction of bioactive compounds:
To separate the bioactive components from the culture broth, a method known as solvent extraction was used. The soup was separated by centrifuging it, and the cell-free supernatant fraction was thoroughly mixed with ethanol at a ratio of 1:1 before being left undisturbed for three to four hours in a separate funnel. After the solvent extraction, the solvent phase was collected and allowed to evaporate in a water bath at a temperature of 60 degrees Celsius for one hour. The material that was produced was characterized by its black hue and its dry nature. Both DMSO and water were able to dissolve the compounds (Dimethyl sulfoxide). The product that was produced as a consequence was collected and put to use in more study (Figure 47).
In previous research, antibiotics from Actinomycetes were extracted using a number of different solvents. These solvents included n-butanol (Sahin and Ugur 2003; Augustine et al., 2005); methanol (Ilicet al., 2005); n-hexane (Beran and Zima 1993); petroleum ether, chloroform, benzene (Thangaduraiet al., 2004); and xylene

Figure 4.38: Extraction of Antimicrobial Compounds

4.7.3 Antibacterial activity of extracted compounds:

Extracts of Streptomyces species HB084 (MS5) and Nocardiopsis species M048 (MS7) from ethyl acetate exhibited considerable antibacterial activity against a variety of bacterial infections. Activity was observed for three different concentrations, including 50 l, 100 l, and 200 l, and the concentration of 200 l showed the maximum antibacterial activity against the test pathogens (Escherichia coli, Staphylococcus aureus, Salmonella typhi, Enterococcus sp., Methicillin Resistant Staphylococcus aureus (MRSA), Bacillus cereus, Klebsiellapneumoniae The Streptomyces sp. HB084 (MS5) isolate had high antibacterial activity against all of the bacterial pathogens that were tested (Plate 5). Although it was less efficient against Enterococcus sp. Klebsiellapneumoniae, Proteus vulgaris, and Streptococcus variants, the isolate Nocardiopsis sp. M048 (MS7) was quite successful against E. coli, Bacillus cereus, and Shigella flexineri. It demonstrated an ability to withstand infection from MRSA, Salmonella typhi, and Staphylococcus aureus. Ali Ramazan et al., 2013 conducted an investigation on the antibiotic manufacturing capabilities of Streptomyces sp., which were shown to be effective against four different types of bacteria: Escherichia coli, Salmonella typhi, Staphylococcus aureus, and Pseudomonas aeruginosa. In a separate study conducted by Nupur Mathuret al. in 2015, it was found that the Streptomyces sp. isolates tested positive for antibacterial activity against both Gram-negative and Gram-positive bacteria.

4.7.4 Antifungal activity of extracted compounds:

There was also an examination of the antifungal activity of a purified substance isolated from an ethyl acetate extract of the Actinomycete isolates Streptomyces sp. HB084 (MS5) and Nocardiopsis sp. M048 (MS7). To name a few: Aspergillus niger, Aspergillus flavus, Candida albicans, Malassezia furfur, and Penicillium Significant action against the test strains

was shown at all three concentrations (50 l, 100 l, and 200 l) where antifungal activity was identified.

Plate 5: Antibacterial activity of the Extracted Compounds of the isolate Streptomyces sp. 118084 (MS5) (Plates showing the zone of Inhibition):

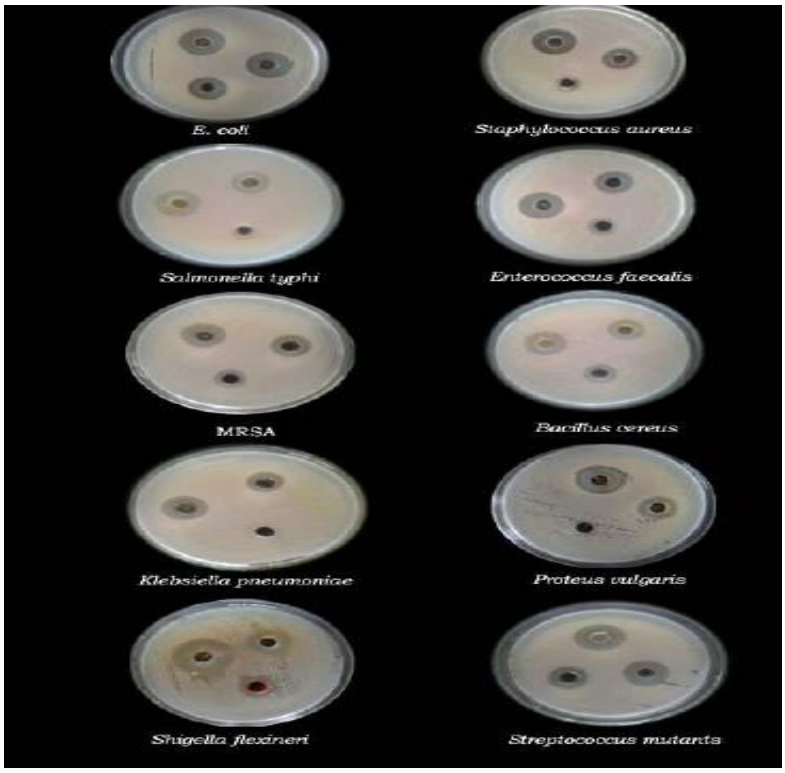
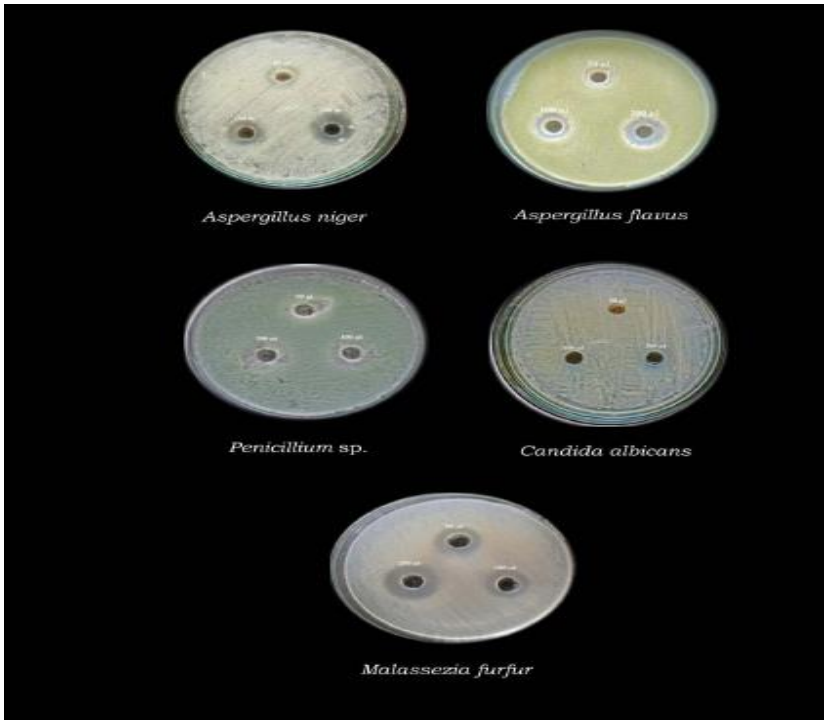


Plate 6: Antifungal activity of the Extracted compound of the isolate Nocardiopsis Sp. M048 (MS7) (Plates showing the zone of Inhibition):



No significant action against the investigated fungal infections was observed for Streptomyces sp. HB084 (MS5). Nocardiopsis sp. M048 (MS7), on the other hand, demonstrated strong activity against Penicillium sp. and considerable activity against all the other chosen fungal infections (Plate 6) High antifungal activity against Aspergillus flavus, Aspergillus niger, and Candida albicans was observed in the ethyl acetate extract of Nocardiopsis sp. VITSVK 5 by Vimal et al., 2009. Similarly, Ferial Rashad et al., 2015 found that Streptomyces fluvissimus FHM275 had wide spectrum efficacy against fungal infections including Candida albicans and Aspergillus niger.

4.8 Invitro Cytotoxicity study-1:

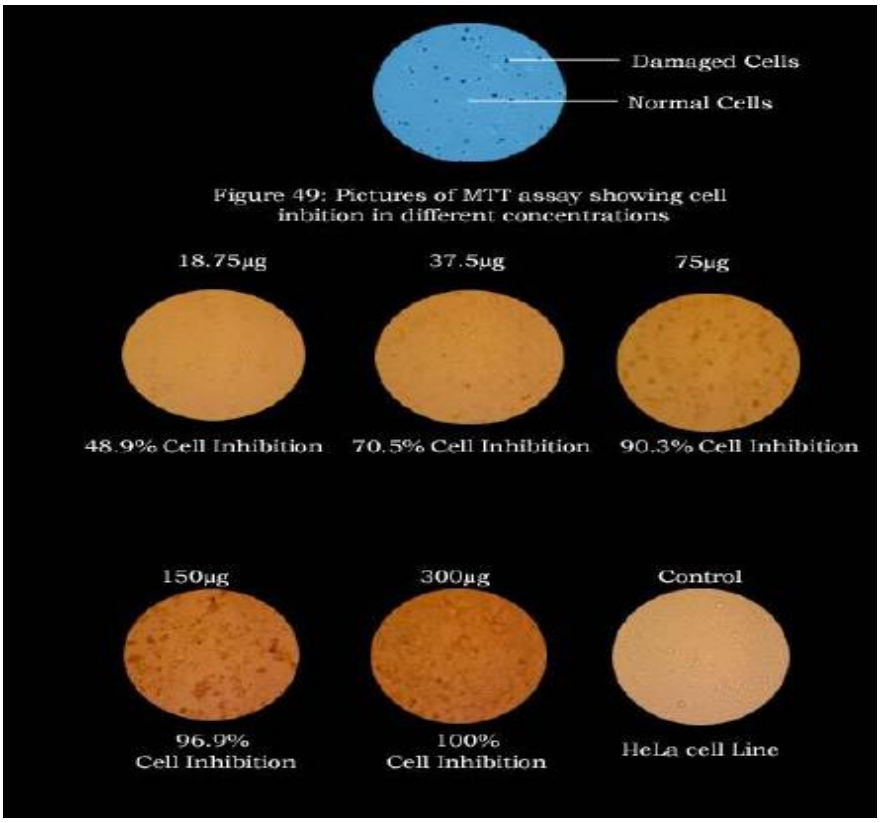
4.8.1 Trypan-blue Exclusion method:

The DLA (Dalton's Lymphoma Ascites) assay was performed on the isolates of Streptomyces sp. HB084 (MS5) and Nocardiopsis sp. M048 (MS7). It was discovered that the concentration of the substance employed led to an increase in the number of cells that had died as a result of their exposure to it. The formula, which was used to compute the percentage of cytotoxicity, is as follows:

$$\%\text{Cytotoxicity} = \frac{\text{Numberofdeadcells}}{\text{Numberoflivingcells+Numberofdeadcells}} X100$$

The isolate Nocardiopsis sp. M048 (MS7) strain did not show any activity, whereas Streptomyces sp. HB084 (MS5) showed action with 90% of cell death in 200 ug of material (Table 12). (Table 12). The cytotoxic effect of Streptomyces sp. extracts increased according to the increase in the concentration of the extract used, according to study conducted by Fathey El-Taweele et al. in 2014. Additionally, he highlighted the fact that Streptomyces roseoflavus may only have a maximum of 75% cytotoxicity.

Figure 4.39. DLA assay results showing damaged cells stained with Trypan Blue Stain



Out of two samples, MS5Streptomyces sp. HB084 (MS5) isolate showed 90% anticancer activity. So, it was selected for another cytotoxicity assay called MTT assay and also for further studies.

4.8.2 Invitro cytotoxicity assay-2:

MTT Assay (Subramanian DeepikaPriyadharshiniet al., 2013):

The formula that was utilized in this experiment to estimate cytotoxic activity is % Cell inhibition = 100 - Abs (sample) / Abs (control) x 100, which was derived from a study that was conducted by Sanjay Patel and colleagues (2009).

The cytotoxic effect that the crude extract of MS5 has on MCF-7 cells (a human breast cancer cell line) is depending on both the quantity of the extract and the amount of time it is exposed to it. The IC50 value for MCF-7 cells was determined to be 19.84 g/ml after extensive research. At a concentration of 37.5 g/ml, it was observed that more than 70 percent of the cells died. At a concentration of 75 g/ml, the death of more than 90% of the cells was observed, and at a concentration of 150 g/ml, 97% of the cells died. The conclusion that can be drawn from these findings is that the crude extract is toxic to MCF-7 cells but has a lower risk of injury to normal cells (Figure 49). The findings of the current study are comparable to those of Sudha Sri Kesavan's (2015) report on the cytotoxic effects of Streptomyces globisporous SU7 against MCF-7 and HeLa cell lines. That report demonstrated that the crude extract was highly toxic to both cell lines, with minimum IC50 values of 19.95 g/ml and 25.1 g/ml, respectively. The findings of the current study are consistent with those of the report. In related research, Vidhyashree and Yamini Sudha Lakshmi (2015) demonstrated that in vitro cytotoxicity of silver nanoparticles against MCF cell lines could be observed when manufactured silver nanoparticles of Streptomyces althioticus were used to treat MCF cell line. This was accomplished by treating MCF cell line with silver nanoparticles produced by Streptomyces althioticus.

Table 4.10: Cell death in different drug concentrations (DLA Assay)

Drug concentration µg/ml	Percentage of cell death(DLA)	
	MS5	MS7
200	90%	_
150	75%	_
100	55%	_
50	22%	_

4.8.3 Antioxidant Activity:

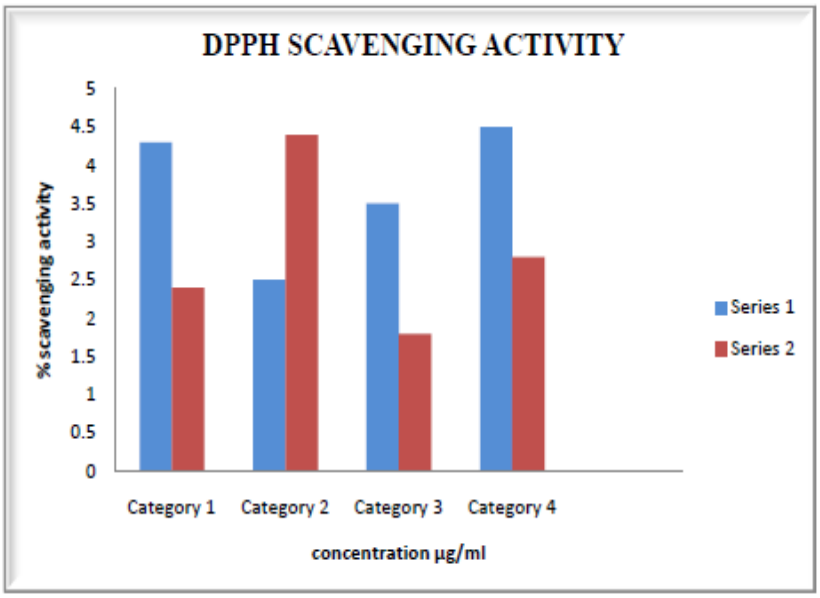
DPPH Radical Scavenging Activity of the isolate MS5:

The DPPH free radical scavenging assay was used to compare the levels of free radical scavenging activity shown by varying doses of solvent extract and ascorbic acid (the

standard). Both the extract and the standard demonstrated a dose-dependent ability to scavenge the free radical known as DPPH* by converting into DPPH. The activity of ascorbic acid in removing free radicals was found to be higher than that of solvent extract. The DPPH• free radical scavenging activity of the ethyl acetate extract of the isolate Streptomyces sp. HB084 (MS5) was determined to be 61.82%, whereas that of standard ascorbic acid was determined to be 79.99% at a concentration of 5 mg/ml (Figure 50).

In a prior investigation, Praveen Kumar et al., 2010 shown that the ethyl acetate extract of Streptomyces species was able to scavenge DPPH free radicals in a dose-dependent manner. In this particular investigation, it was discovered that the scavenging activity of extract is dosage dependent, which means that the higher the concentration, the greater the scavenging activity. In spite of the fact that the extract's DPPH radical scavenging abilities were lower than those of ascorbic acid, the study demonstrated that the extract has the ability to donate proton and has the potential to act as free radical inhibitors or scavengers, possibly performing the role of primary antioxidants. The formation of the ferrous (Fe2+) form is brought about by the presence of reductants in the samples, such as antioxidant compounds. This results in the reduction of the ferric form, Fe3+/ferricyanide.

Figure 4.40: DPPH Radical Scavenging Activity of the isolate Streptomyces sp. HB084 (MS5)



As a result, Fe2+ may be measured by observing the production of Perl's Prussian blue at a wavelength of 515 nm (Chang et al., 2002).

It is possible that the ability of a chemical to reduce other substances may serve as a substantial signal of the potential antioxidant activity of that molecule (Meir et al., 1995). Nevertheless, the antioxidant activity of putative antioxidants has been attributed to a number of different mechanisms. Some of these mechanisms include the prevention of chain initiation, the binding of transition metal ion catalysts, the decomposition of peroxides, the prevention of continued hydrogen abstraction, and radical scavenging (Diplock, 1997). Chidambara-Raja et al., 2012 also revealed the powerful antioxidant and cytotoxic effects of an ethyl acetat extract of Streptomyces sp. ngp1 that was isolated from the western coast of India.

4.9 Separation of Secondary metabolites:

4.9.1 Column Chromatography:

Column chromatography was used for the purification procedure, and the combination of ethanol and water that was used for elution had a volumetric ratio of 50:50 by volume. After eluting the MS5 culture extract via a column, a total of 30 distinct fractions were obtained. Antimicrobial activity was determined for each of the different fractions that were collected. Antimicrobial activity was discovered at fractions ranging from 16 to 23, with the highest level of activity identified at fraction 21. Through the use of thin-layer chromatography, the active fraction was refined even more.

Similar findings were found in earlier research conducted by Afifie et al., 2012, who purified the bioactive substance using column chromatography packed with silica gel. The substance was eluted with a mixture of chloroform and methanol that had an 8:2 volume-to-volume ratio, and the results showed that the bioactivity was at its highest between fraction numbers 14 and 23. The bioactive substance was purified by Houssam Atta, 2015, using column chromatography packed with silica gel. It was eluted with chloroform and methanol (24:1, v/v), and the results showed that the fraction numbers 14 to 23 had the highest level of activity.

4.9.2 Thin Layer Chromatography (TLC):

TLC was used to further purify the secondary metabolites of the eluant that had the highest level of antibacterial activity. Iodine vapour was allowed to come into contact with the plates, and a single area that was dark brown in color was detected. The retention factor, denoted by the acronym Rf, of relocated spot was 0.9. Augustine et al., 2006 purified the bioactive substance of Streptomyces albidoflavus PU23 by using the technique of thin-layer chromatography, and they found that the Rf value of the active fraction was 0.78. These results were reported in earlier studies that were conducted by Augustine et al., 2006. In 2014, Prakasham Reddy Shetty and colleagues used the Thin Layer Chromatography method to isolate the bioactive material produced by Streptomyces parvulus. They determined that the Rf value of the active fraction was 0.20.

4.9.3 Identification of Bioactive Compounds:

Utilizing Fourier transform infrared and gas chromatography mass spectrometry, we were able to identify and characterize the bioactive chemicals found in the successful isolation of Streptomyces sp. HB084 (MS5).

Fourier Transform Infrared Spectrophotometer (FTIR):

The FTIR technique was used in order to determine the functional groups that were present in the anti-microbial compounds. The alcohols and phenols group were found to be present with O-H stretching when the frequency was measured at 3410 cm-1. There were alkanes with C-H stretch when looking at the frequency at 2854 cm-1. The frequency of 1728 cm-1 indicated the presence of aldehydes with a C=O stretch. The frequency at 1103 cm-1 indicated the presence of carboxylic acids with a C-O stretch. The frequency of 802 cm-1 demonstrated the presence of alkyl halides with C-Cl stretch. The frequency of alkyl halides with C-Br stretch was observed at 671 cm-1 and 601 cm-1. Figure 51 displays the spectrum data that were obtained from the FTIR study.

A similar type of result was obtained by NupurMathuret al., 2015, when they tested the FTIR spectrum of an ethyl acetate extract of Streptomyces sp. that exhibited an absorption band at 600-800 cm-1, which indicated Alkyl halide groups with C-Cl stretch. NupurMathuret al.,

2015, was published in the journal Microbiology. The FTIR spectra of the ethyl acetate extract of Streptomyces atrovirensH33 was obtained by AlaaEldinHosnyet al., 2015, and it displayed absorption bands at 2924 cm-1. These bands suggested the presence of an aldehyde group with C=O stretch. The FTIR spectrum study that Zarina and anima Nanda, 2014 performed for silver nanoparticles made using Streptomyces albaduncus showed an absorption band at 3273.20 cm-1. This band suggested the presence of an alcohol and phenol group with O-H stretch.

Challenges and Future Prospects:

The final section of the review highlights the challenges faced in developing heterocyclic compounds as anti-bacterial agents. It addresses issues like toxicity, bioavailability, and potential resistance development. Additionally, the review outlines possible future research directions, including the combination of heterocyclic compounds with existing antibiotics, the exploration of natural sources, and the incorporation of nanotechnology for enhanced delivery.

Conclusion:

In conclusion, the synthesis of heterocyclic compounds and their evaluation for anti-bacterial activities presents a promising and dynamic field in the quest for novel antibacterial agents. The diverse range of heterocyclic scaffolds and their versatility in synthetic approaches have allowed researchers to explore a vast chemical space for potential antimicrobial candidates. Understanding the mechanisms by which these compounds exert their antibacterial effects is critical for optimizing their activity and minimizing the risk of resistance development. Structure-activity relationship studies have proven instrumental in guiding rational design strategies, leading to the development of more potent and selective antibacterial agents. Despite significant progress, challenges such as toxicity, bioavailability, and resistance still persist, necessitating continuous efforts to overcome these obstacles. The collaboration of multidisciplinary approaches, including the combination of heterocyclic compounds with existing antibiotics and innovative drug delivery systems, holds the potential to revolutionize antibacterial therapy and address the growing global threat of antibiotic resistance. With further research and innovation, the synthesis of heterocyclic compounds as antibacterial agents will undoubtedly continue to contribute significantly to improving public health and combating infectious diseases caused by drug-resistant bacteria.

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