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# Synthesis, Characterization, and Biological Evaluation of Benzimidazole Derivatives as Antimicrobial

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**ABSTRACT**

Benzimidazole is a frequently used chemical reagent in the manufacture of antifungal, anthelmintic, and antibacterial medications. Heterocyclic aromatic compounds can be found in the chemical reagent benzimidazole. In this study, we synthesised benzimidazole derivatives from diamino toluene. The synthesised derivatives are identified using Fourier Transform Infrared Spectroscopy (FT-IR), which exhibits the spectrum of the functional group of benzimidazole at (N-H str) at 3256 cm-1, (C-N str) at 1256 cm-1, (C=C str) of the benzene ring at 1656 and 1443 cm-1, and (C=C str) of the alkene at 1379 cm-1. The IR spectra of the end chemical and the substrate were examined, and it was found that the distinctive peak of the aldehyde (C=O) was eliminated as a result of the reaction. Disc-diffusion methods were used to test the product's antibacterial activity against the bacterial strains Bacillus cereus, Proteus vulgaris, Klebsiella pneumonia, and Enterococcus faecalis and its antifungal activity against the fungi Aspergillus niger and Aspergillus fumigatus at a concentration of 200 mg/m. These results suggest that imidazole derivatives are more effective antibacterial and antifungal agents than traditional antibacterial and antifungal drugs. Benzimidazole derivatives can both prevent and treat a variety of bacterial and fungal diseases.

## INTRODUCTION:

A wide range of parasitic bacteria, such as S. pyogenes, Staphylococcus aureus, Escherichia coli, and Salmonella typhimurium, have a substantial impact on the health of human mucosal tissues. Infections with E. coli, Salmonella typhimurium, S. aureus, and S. pyogenes can result in serious host tissue damage and potentially serious diseases. These bacterial parasites cause millions of people in developing countries to experience food poisoning, rheumatic fever, and diarrhoea. [1] More than 50 million individuals are affected worldwide, and up to 1, 10,000 of them die every year. Amoxicillin, norfloxacin, and ciprofloxacin are the most frequently prescribed medications for this bacterial infection; nevertheless, they have substantial adverse effects. [2] If the frequency of infections brought on by bacteria resistant to one or more antibiotic classes keeps increasing, there is a substantial risk of treatment failures and issues. [3,4] As a result, numerous research teams have invested a lot of time and energy in the quest for new antimicrobial medications.

Benzimidazoles and their analogues are well-known physiologically active N-containing heterocycles with a wide range of biological activity. [5, 6, 7, 8, 9, 10, 11, 12] On the other hand, from a pharmaceutical standpoint, pyrazole and its derivatives are one of the most important classes of organic heterocyclic compounds. These substances contain antiviral, herbicidal, antibacterial, and antifungal activities. [13,14,15,16] Several of its compounds have been found to have notable hypoglycemic, anti-arrhythmic, sedative, and anti-inflammatory activities. [17, 18, 19, 20] Numerous of its constituents have been discovered to possess noteworthy hypoglycemic, anti-arrhythmic, sedative, and anti-inflammatory properties. [17, 18, 19, 20] In light of these findings and as part of our ongoing drug research programme aimed at the creation of new, safer, and more biologically active compounds in search of more potent and benign antimicrobial agents, it was of interest to synthesise a new series of benzimidazole-coupled pyrazole derivatives.

## EXPERIMENTAL:

**Materials and Methods:** In this experiment, analytical-grade solvents were used. The purity of the items was evaluated using TLC plates, and their composition was identified using melting point apparatus. N-hexane and ethyl acetate were frequently used as the solvent medium for monitoring reactions on TLC plates. The course of the reaction was monitored using thin layer chromatography. As a visualisation aid, a UV light was used.

The entire process was carried out in clean glassware under specified catalyst conditions that may be either basic or acidic. Different spectroscopic methods, including 1H NMR and 13C NMR, were used to describe all produced molecules. At 300, 400, and 500 MHz, the Advance Bruker AM was used for 1H NMR investigations. Thin Layer Chromatography (TLC) was performed on pre-coated silica gel aluminum plates with dimension 3x8 cm (Kieselgel 60, 254, E. Merck, Germany). The chromatogram was visualized with dual wavelength by UV at 254 and 365 nm. Melting point was found out on Gallon kemp apparatus

**SYNTHESIS:**

**STEP 1.**

**Synthesis of 2-ethyl 5-methyl benzimidazole:**

1. This is the first step towards the synthesis of substituted hydrazone.

2. First potassium hydroxide was taken in 100 mL round bottom flask along with ethanol and stirred on hotplate until KOH was dissolved.

3. After this di amino toluene was added to the mixture with constant stirring.

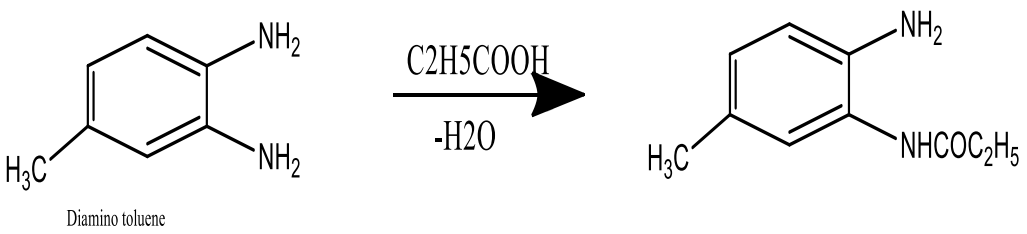
4. Then Propionic acid was added drop wise and stirred for 2 hr the temperature of reaction 90 ˚C. Progress of reaction was monitored by TLC.

5. After 2 hr when the reaction is completed. The whole mixture keep in ice cold water.

6. Then the benzimidazole is slightly started to crystallized and in 15 min. the whole product is separated.

7. The product was removed by filtering the whole mixture and filtrate was kept for whole night.

8. Ethanol was evaporated and gets shiny white needle like crystals of 2-ethyl 5-methyl benzimidazole.




Diamino toluene

**Physical data**

- Shiny needle like white crystals
- Chemical Formula: $C_{10}H_{14}N_2O$
- Molecular Weight: 178.23
- Melting Point: 150 ˚C
- Yield: 90%

**Step 2**

**Synthesis of 2-ethyl 5-methyl benzimidazole:**

1. After this di amino toluene was added to the mixture with constant stirring.

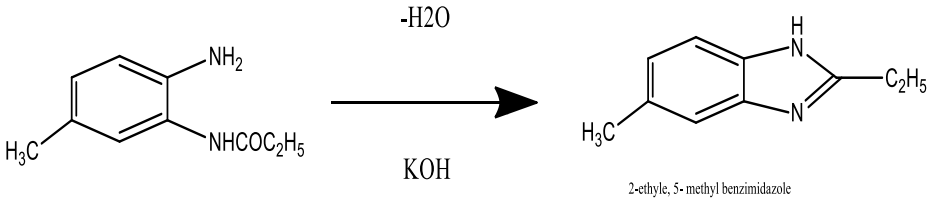
2. Then Propionic acid was added drop wise and stirred for 2 the temperature of reaction 90 ˚C. Progress of reaction was monitored by TLC.

3. After 2 hr when the reaction is completed. The whole mixture keep in ice cold water.

4. Then the benzimidazole is slightly started to crystallized and in 15 min. the whole product is separated.

5. The product was removed by filtering the whole mixture and filtrate was kept for whole night.

6. Ethanol was evaporated and gets shiny white needle-like crystals of 2-ethyl 5-methyl benzimidazole.




**Physical data**

- Shiny needle like white crystals
- Chemical Formula: C10H12N2
- Molecular Weight: 160.22

- Melting Point: 150 ˚C
- Yield: 90%

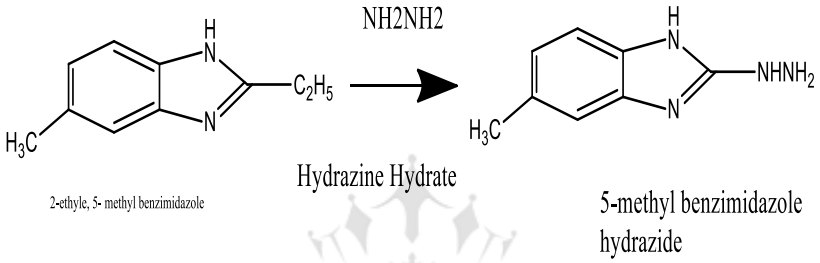
**Step 3.**

**Synthesis of 2-ethylbenzimidazol hyrazide:**

1. In third step, 2-ethyl 5-methyl benzimidazole was refluxed in methanol with hydrazine hydrate for about 10h.

2. The product, 2-ethylbenzimidazol hyrazide get was poured into ice cold water until precipitate was formed.

3. Precipitate was filtered and then dried in open atmosphere.




**Physical data:**

- Compound was creamy coloured.
- Chemical Formula: C8H10N4
- Molecular Weight: 162.19
- Melting Point: 85 ˚C
- Yield: 84%

**Step 4**

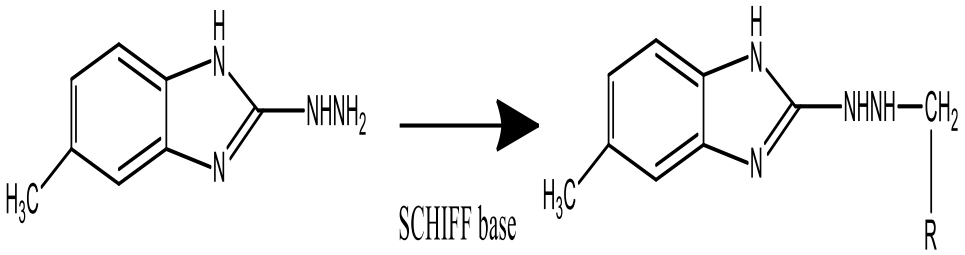
**Synthesis of substituted hydrazine using different aldehydes:**

1. In fourth step, 5-methyl benzimidazole hydrazide was dissolved in methanol in RB with 2-3 drops of acetic acid on hotplate.

2. After 10 minutes aldehydes was added and refluxed the whole mixture for about 5-6 hours. Progress of the reaction was monitored by TLC.

3. After completion of reaction, the mixture was poured into ice cold water until precipitate was formed.

4. Precipitate was collected by filtration, washed with water and then dried in open atmosphere.




**Physical data**

- Compound was creamy coloured.
- Yield: 84%

**SCHEME**

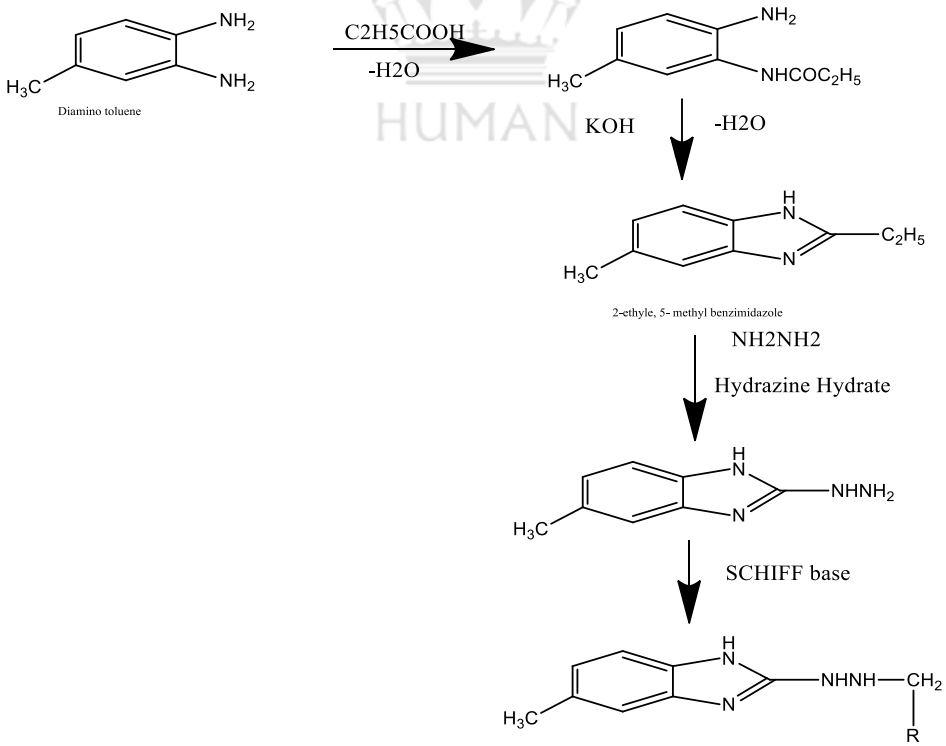
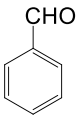
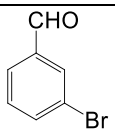
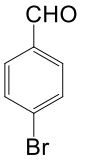
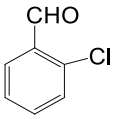
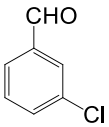
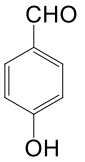
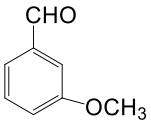
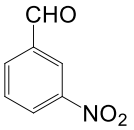
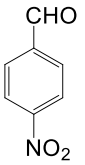
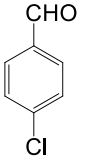


**Table 1:- Lists of Aldehydes**

| Sr.No. | R - Aldehyde Name | Structure |
|---|---|---|
| Comp -I | Benzaldehyde |  |
| Comp -II | 3-Bromo Benzaldehyde |  |
| Comp -III | 4-Bromo Benzaldehyde |  |
| Comp -IV | 2-Chloro Benzaldehyde |  |
| Comp –V | 3-Chloro Benzaldehyde |  |
| Comp –VI | 4-Hydroxy Benzaldehyde |  |
| Comp –VII | 3-methoxy Benzaldehyde |  |
| Comp –VIII | 3-Nitro Benzaldehyde |  |
| Comp –IX | 4-Nitro Benzaldehyde |  |
| Comp -X | 4-Chloro Benzaldehyde |  |

## SCREENING OF ANTI-MICROBIAL ACTIVITY

**Screening of anti-bacterial activity**: The gram-positive bacteria Bacillus cereus NL98 and Enterococcus faecium ATCC 29212 and the gram-negative bacteria Proteus vulgaris NCTC 4635 and Klesibella pneumonia ATCC 29655 were used to investigate the synthesised compounds' in vitro anti-bacterial activity. Nosocomial infections, biliary tract infections, and urinary tract infections are frequently brought on by these drugs.

The gram-negative bacterium Klesibella pneumonia causes infections of the bronchitis, pneumonia, and bronchopneumonia. The gram-positive bacteria Bacillus cereus and Enterococcus faecalis cause the illnesses endocarditis, bacteremia, meningitis, and septicemia. The results gathered indicated that every drug under investigation has anti-bacterial activity against both gramme positive and gramme negative pathogens.

The CMP 4 displayed the following sequence of important activity: Klebsiella pneumonia > Proteus vulgaris > Enterococcus faecium > Bacillus cereus The significant action on the CMP 10 was displayed in the following order: Enterococcus faecalis > Klebsiella pneumonia > Proteus vulgaris > Bacillus cereus.

The following sequence is when the chemical CMP 4 shown substantial activity: The Proteus vulgaris Bacillus cereus > Enterococcus faecium>Klesibella pneumonia. The CMP 10 displayed the following sequence of important activity: Proteus vulgaris, Bacillus cereus, Enterococcus faecium, and Klesibella pneumonia.

In the order Klesibella pneumonia > Bacillus cereus > Enterococcus faecium > Proteus vulgaris, the CMP 4 shown substantial action. Proteus vulgaris was more effectively inhibited by CMP 10. Bacillus cereus was more effectively inhibited by CMP 4. Proteus vulgaris showed stronger activity in response to CMP 10.

Klesibella pneumonia, a gram-negative bacterium, causes bronchitis, pneumonia, and bronchopneumonia infections. The gram-positive bacteria Bacillus cereus and Enterococcus faecium cause endocarditis, bacteremia, meningitis, and septicemia, among other illnesses. According to the data gathered, every drug under investigation was found to exhibit anti-bacterial effect against both gramme positive and gramme negative organisms.

**Table 2: In vitro anti-bacterial activity of synthesized compounds by disc diffusion method**

| Bacteria Compound | Bacillus cereus | Proteus vulgaris | Klebsiella pneumonia | Enterococcus faecium |
|---|---|---|---|---|
| CMP-1 | 15 | 16 | 14 | 17 |
| CMP-2 | 17 | 18 | 15 | 17 |
| CMP-3 | 17 | 16 | 15 | 16 |
| CMP-4 | 25.5 | 26 | 24 | 23 |
| CMP-5 | 12 | 14 | 13 | 10 |
| CMP-6 | 19 | 20 | 21 | 19 |
| CMP-7 | 10 | 12 | 11 | 13 |
| CMP-8 | 14.8 | 15 | 16 | 13 |
| CMP-9 | 18 | 20 | 19 | 17 |
| CMP-10 | 26 | 25 | 24 | 27 |
| Ciprofloxacin | 30 | 30 | 30 | 30 |

**Screening of anti-fungal activity:**

The two fungus Aspergillus niger and Aspergillus fumigatus were used to test the synthetic compounds' in-vitro anti-fungal efficacy. These are the germs that cause bronchitis, aspergillosis, and serious lung infections.

The information showed that each chemical tested exhibited anti-fungal properties. However, CMP 4 and CMP 10 stood out among all the chemicals generated for their potent anti-fungal activity against both fungi.

However, it was shown that the anti-fungal activity of CMP 1, 2, and 3 against the tested organism was less effective than that of the anti-fungal drug ketoconazole at the tested dose level.

**Table 3: In vitro anti-fungal activity of synthesized compounds by disc diffusion method**

| Fungi Compound | Aspergillus niger | Aspergillus fumigates |
|---|---|---|
| CMP-1 | 15 | 18 |
| CMP-2 | 17 | 20 |
| CMP-3 | 19 | 17 |
| CMP-4 | 22 | 22 |
| CMP-5 | 15 | 19 |
| CMP-6 | 20 | 23 |
| CMP-7 | 14 | 16 |
| CMP-8 | 12 | 13 |
| CMP-9 | 16 | 17 |
| CMP-10 | 22 | 25 |
| KETOCONAZOLE | 30 | 30 |

## RESULT AND CONCLUSION

In recent years, increased focus has been placed on the production of benzimidazole derivatives as a source of novel anti-microbial medications. New benzimidazole derivative development is a major focus of drug research. In an effort to expand the category of benzimidazole derivatives, we developed a variety of novel molecules that include the benzimidazole ring. The O-phenylene diamine reacted with the appropriate carboxylic acid to form the corresponding benzimidazole in a respectable yield under challenging dehydration reaction circumstances. The same method was carried out using microwave irradiation to produce the matching benzimidazole in excellent yields.

Then, a variety of 5-nitro substituted benzimidazole derivatives were made using concentrated HNO3 and concentrated H2SO4. Then, 5-nitro substituted benzimidazoles were reduced with a Zn/NaOH solution to yield 5-amino substituted benzimidazoles.

The purity of the created compounds was assessed using TLC (Rf) and melting point calculations. Since our identified compounds were known to have an anti-microbial effect, the produced compounds were examined for their anti-bacterial and anti-fungal properties.

The structure of the generated compounds was ascertained using spectral (IR, and 1H NMR) analytical data. The NH band (3463-3114 cm-1) and NH proton signal (5.0 ppm) of 2-substituted benzimidazole in the IR and 1H NMR spectra, respectively, of 137 of the generated compounds (CMP 1 to CMP 10) confirmed the synthesis of benzimidazole nucleus.

Additionally, the major amino group N-H bending was associated with a substantial signal between 1648 and 1622 cm-1. The presence of the primary amino group was confirmed by a singlet at 3.48 for two protons in CMP 1-CMP 10. Additional proof that the identified compounds had the expected chemical structure was supplied by the fragmentation peaks. With regard to the particular group of bacteria that were the focus of the investigation, all synthesised substances were successful. Because fewer species have been used in this experiment, it is warranted to screen these chemicals utilising a variety of species and resistant strains. Each substance demonstrated remarkable anti-bacterial and anti-fungal activity even at lower concentrations.

The findings show that compound CMP 4 is a very strong candidate for anti-bacterial studies and that compound CMP 10 is a much stronger possibility for anti-fungal studies. Although the examined substances' antimicrobial activity was inferior to that of their traditional counterparts, in this case the antifungal medicine ketoconazole and the antibacterial drug ciprofloxacin, the tested substances were nevertheless active against microorganisms.

**REFERENCES:**

1. Puertoa SA, Fernandeza GJ, Castillob LDJ, Jose M, Pinoa S, Anguloa PG. In vitro activity of β-lactam and non β-lactam antibiotics in extended spectrum β-lactamase producing clinical isolates of Escherichia coli. DiagnMicrobiol Infect Dis. 2006;54:135–9.
2. Nolan CM, Chalhub GE, Nash GD, Yamauchi T. Treatment of bacterial meningitis with intravenous Amoxicillin. Antimicrob Agents Chemother. 1979;16:171–5.
3. Beekmann SE, Heilmann KP, Richter SS, Garcia-de-Lomas J, Doern GV. Antimicrobial resistance in Streptococcus pneumoniae, Haemophilusinfluenzae, Moraxella catarrhalis and group A β-haemolytic Streptococci in 2002-2003: Results of the multinational GRASP surveillance program. Int J Antimicrob Agents. 2005;25:148–56.
4. Spellberg B, Guidos R, Gilbert D, Bradley J, Boucher H, Scheld WT, et al. The epidemic of antibiotic resistant infections: A call to action for the medical community from the infectious diseases society of America. Clin Infect Dis. 2008;46:155–64.
5. Preston PN. Synthesis, reactions and spectroscopic properties of benzimidazoles. Chem Rev. 1974;74:279–314.
6. Kazimierczuk Z, Andrzejewska M, Kaustova J, Klimesova V. Synthesis and antimycobacterial activity of 2-substituted halogenobenzimidazoles. Eur J Med Chem. 2005;40:203–8.
7. Vinodkumar R, Vaidya SD, Siva Kumar BV, Bhise UN, Bhirud SB, Mashelkar UC. Synthesis, antibacterial, antiasthmatic and antidiabetic activities of novel N-substituted-2-(4-phenylethynyl-phenyl)-1H-

benzimidazoles and N-substituted-2[4-(4,4-dimethyl-thio chroman-6-yl- ethynyl)-phenyl)-1H-benzimidazoles. Eur J Med Chem. 2008;43:986–95.
8. Yun H, Baogen W, Yang J, Robinson D, Risen L, Ranken R, et al. 2-Piperidin-4-ylbenzimidazoles with broad spectrum antibacterial activities. Bioorg Med ChemLett. 2003;13:3253–6.
9. Ozkay Y, Tunali Y, Karaca H, Isikdag I. Antimicrobial activity and a SAR study of some novel benzimidazole derivatives bearing hydrazones moiety. Eur J Med Chem. 2010;45:3293–8.
10. Grassi A, Ippen J, Bruno M, Thomas G, Bay P. A thiazolylaminobenzimidazole derivative with gastro protective properties in the rat. Eur J Pharmacol. 1991;195:251–9.
11. Ayhan-Kilcigil G, Altanlar N. Synthesis and antimicrobial activities of some new benzimidazole derivatives. Farmaco. 2003;58:1345–50.
12. Boiani M, Gonzalez M. Imidazole and benzimidazole derivatives as chemotherapeutic agents. Mini Rev Med Chem. 2005;5:409–24.
13. Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in food – areview. International Journal of Food Microbiology, 94, 223-253.
14. David, W., Zhijan, K., & Christine, C. (2004). Synthesis of dicationic extended bis-benzimidazoles. Molecules, 9, 158-163.
15. Vimol, S., Chanwit, T., Veena, N., Nuntavan, B., Kulkanya, C., Siwimol, P., et al. (2012). Antibacterial activity of essential oils from Citrus hystrix (makrut lime) against respiratory tract pathogens. Science Asia, 38, 212-217.
16. Burt, S. J. (2004). Essential oils: Their antibacterial properties and potential applications in food—a review. International Journal of Food Microbiology, 94, 223-253.
17. Kim, J., Marshall, M. R., & Wei, C. I. (1995). Antibacterial activity of some essential oil componentsagainst five foodborne pathogens. Journal of Agricultural and Food Chemistry, 43, 2839-2845
18. Lambert, R. J., Skandamis, P. N., Coote, P. J., &Nychas, G. J. (2001). A study of the minimum inhibitoryconcentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of AppliedMicrobiology, 91, 453-462.
19. Prabuseenivasan, S., Jayakumar, M., &Ignacimuthu, S. (2006). In vitro antibacterial activity of someplant essential oils. BMC Compl Alternative Med, 6, 39-47.
20. Sabulal, B., Dan, M., Pradeep, N. S., Valsamma, R. K., & George, V. (2006). Composition and antimicrobialactivity of essential oil from Amomumcannicarpum. Acta Pharmaceutica, 56, 473-480.
21. Murugulla, A., &Donthabhakthuni, S. (2011). Room temperature synthesis of benzimidazolederivatives using reusable cobalt hydroxide (II) and cobalt oxide (II) as efficient solid catalysts.Tetrahedral Letter, 52, 5575-5580.
22. Nanasombat, S., &Lohasupthawee, P. (2005). Antibacterial activity of crude ethanolic extracts andessential oils of spices against salmonellae and other enterobacteria. KMITL Science and Technology, 5,527-538.