Global Scientific JOURNALS

GSJ: Volume 7, Issue 9, September 2019, Online: ISSN 2320-9186
www.globalscientificjournal.com

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF 2, 5-DIMETHYL-4-METHOXYLBENEZENE THIOACETIC ACID

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ABSTRACT

The resistance of micro-organisms to antibiotics has been a serious challenge facing the health sector since the discovery of penicillin. The research was conducted to develop new organic molecules possessing antimicrobial activities against drug-resistant micro-organisms. p-Xylenol was methylated to give 2, 5-dimethyl Anisole which was converted to 2, 5-dimethyl-4-methoxylbenzenesulphonyl chloride in the presence of chlorosulphonic acid. The compound was reduced to 2, 5-dimethyl-4-methoxylbenzene thiol using zinc and concentrated sulphuric acid. Benzoyl chloride was coupled with the thiol to produce 2, 5-dimethyl-4-methoxylbenzenebenzoyl sulphide. Reaction of 2, 5-dimethyl-4-methoxylbenzene thiol with monochloroacetic acid afforded 2, 5-dimethyl-4-methoxylbenzenethioacetic acid. The thiol was also coupled with acetyl chloride to give 2, 5-dimethyl-4-methoxylbenzeneacetyl chloride. The synthesized compounds were characterized by FTIR and NMR. The screening of the synthesized

compounds for antibacterial and antifungal activities showed that the compounds possess antimicrobial properties.

Keywords: p-Xylenol, bacterial resistance, 2, 5-dimethyl-4-methoxylbenzenethioacetic acid, antimicrobial agents and 2, 5-dimethyl-4-methoxylbenzeneacetyl chloride.

1.0 INTRODUCTION

Micro-organisms have been known to resist the activities of antibiotics since penicillin was discovered [1]. Emergence of microbial resistance to commercial drugs have been increasing at a worrisome rate on a daily basis and attributed to several factors including mutation and enzymatic activities [2, 3]. With the rate at which bacteria develop resistance to approved chemotherapeutic agents, bacterial infections may continue to be leading causes of death globally [4, 5]. Hence, the search for new chemotherapeutic agents remains the heart of modern medicinal chemistry [6]. *Klebsiella pneumoniae, Clostridium difficile,* and *Escherichia coli,* are among the Gram-negative bacteria that have developed resistance to virtually all classes of antibiotics. *Staphylococcus aureus, Streptococcus pneumoniae* and *Pseudomonas aeruginosa* are among the class of Gram-positive bacteria that are difficult to treat because of their resistance to drugs [7]. Hence, microbial infection has significantly contributed to high level of morbidity and mortality worldwide [8, 9]. Development of new antimicrobial agents is required to meet the rising challenge of antimicrobial resistance.

Several benzene-based antimicrobial agents including phthalazinones derivatives (10) and benzene-(1, 4-diimine)-substituted-4,4-10H-diphenothiazine derivatives (11) have been synthesized and they possessed antimicrobial activities. Some benezene derivatives have been reported to exhibit biological activities such as antiparkinsonian, anticonvulsant, antihistaminic,

antihelmatic, antiviral, antiparasitic and CNS depressant (11). However, more is still expected from benzene derivatives as antimicrobial agents. The research was aimed at synthesizing new derivatives of benzene and investigating their antimicrobial activities.

2.0 MATERIALS AND METHODS

All the syntheses were carried out as described by Vogel, 1978 [12].

2.1 Materials

p-Xylenol, sodium hydroxide, dimethyl sulphate, chlorosulphonic acid, monochloroacetic acid, acetyl chloride, and benzoyl chloride were all of analytical grade and used directly. Fourier Transform Infrared (FTIR) spectra were recorded with Cary model 630 spectrophotometer. Proton Nuclear Magnetic Resonance (^{1}H NMR) spectra were obtained on Agilent NMR spectrometer (400 MHz). The chemical shift started from 3.50 ppm instead of 0.00 ppm. Hence, the peaks were assigned by difference as shown below.

$$\delta_H = \delta - 3.50 \text{ ppm} \quad \text{----------(eqn 1)}$$

δ_H = calculated chemical shift

δ = observed chemical shift

2.0 EXPERIMENTAL

2.1 Synthesis of 2, 5-dimethyl anisole

p-Xylenol (10 g, 0.164 mol) was dissolved in a solution of sodium hydroxide (80 ml, 15%). The temperature of the mixture was reduced to 3^{o}C. Dimethyl sulphate (13 ml, 0.206 mol) was added drop-wise for 1 h. The resulting mixture was refluxed in oil-bath for 1 h and cooled. Thereafter, water was added and the oil was extracted with chloroform (20ml X 3). The chloroform layer

was washed with water, dried with magnesium sulphate and distilled to produce 2, 5-dimethyl anisole (**1**). Colourless oil, Yield: 61%, R_f: 0.7 ($C_6H_5CH_3$ and $CHCl_3$, 1:2).

2.2 Synthesis of 2, 5-dimethyl-4-methoxylbenzene sulphonylchloride

2, 5- dimethylanisole (10 g, 0.07 mol) was dissolved in dry chloroform (100ml) in a three necked flask equipped with thermometer, a calcium chloride guarded tube and a dropping funnel. The mixture was cooled in ice-salt mixture and chlorosulphonic acid (38 g, 0.326 mol) was added drop-wise through the dropping funnel with continuous stirring. After 1 h, the mixture was poured into excess crushed ice and the chloroform layer separated. The aqueous layer was extracted with chloroform (20ml) 3 times. The extract was washed with water, dried with anhydrous magnesium sulphate and distilled giving 2, 5-dimethyl-4-methoxylbenzene sulphonylchloride (**2**). White crystal, Yield: 93.3%, m. p. 98^oC, (lit m.p. 98^oC).

2.3 Synthesis of 2,5-dimethyl-4-methoxylbenzene thiol

Concentrated sulphuric acid (10 ml, 0.187 mol) was dissolved in crushed ice (20 g) and 2, 5-dimethyl-4-methoxylbenzene sulphonylchloride (2 g, 0.012 mol) was dissolved in it. The resulting mixture was heated on a water bath and zinc dust (5 g) was added gradually for over 2 h. Concentrated sulphuric acid (10 ml, 0.187 mol) was further added for over 2 h and heated at 95-100^oC for 8 h. After cooling, distilled water (100 ml) was added to dissolve the entire solid and it was extracted with chloroform (25 ml X 3). The extract was washed with water, dried over magnesium sulphate and distilled. 2, 5-dimethyl-4-methoxylbenzene thiol (**3**) produced was purified by column chromatography (mobile phase: benzene). Yellow oil, Yield: 20%, R_f: 0.9 ($C_6H_5CH_3$ and $CHCl_3$, 1:1), FTIR (KBr, cm^{-1}): 2918.5 (sp^3 C-H), 3004.2 (Ar-C-H, stretching vibration), 834.8 (Ar-C-H, bending vibration), 1602.8 (Ar-C=C-C), 1054.8 (C-O-C) and 2728.4

(S-H). δ_H ($CdCl_3$, δ -3.5, ppm): 7.01 (1H, s, Ar-H), 6.75 (1H, s, Ar-H), 6.67 (1H, s, Ar-H), 3.79 (3H, s, -OCH_3), 2.32 (3H, s, Ar-CH_3) and 2.12 (3H, s, Ar-CH_3).

2.4 Synthesis of 2, 5-dimethyl-4-methoxylbenzenebenzoyl sulphide

Chloroform (20 ml) was measured into conical flask, triethyl amine (1 ml) was added and shaken. 2, 5-dimethyl-4-methoxylbenzene thiol (0.504 g, 0.003 mol) dissolved in the mixture was reacted with benzoylchloride (0.423 g, 0.003 mol) and stirred for 6 h at ambient temperature. After that, the resulting mixture was transferred into separating funnel; water (50 ml) was added to separate out the mixture and extracted with chloroform (20ml X 3). The extract was dried over magnesium sulphate and distilled to give 2, 5-dimethyl-4-methoxylbenzenebenzoyl sulphide (**4**). Compound **4** was purified by column chromatography (mobile phase: ethyl acetate). Brown liquid, Yield: 40%, R_f: 0.83 ($C_6H_5CH_3$ and $CHCl_3$, 1:1), FTIR (KBr, cm^{-1}): 2922.2 (sp^3 C-H), 842.4 (Ar-C-H, bending vibration), 1602.8 (Ar-C=C-C), 1051.1 (C-O-C), 738 (C-S) and 1718.3 (C=O). δ_H ($CdCl_3$, δ -3.5, ppm): 7.20 (1H, d, Ar-H), 7.64 (2H, m, Ar-H), 3.73 (3H, m, -OCH_3), 2.79 (3H, m, Ar-CH_3) and 3.40 (3H, m, Ar-CH_3).

2.5 Synthesis of 2, 5-dimethyl-4-methoxylbenzene thioacetic acid

2, 5-dimethyl-4-methoxylbenzene thiol, (0.504 g, 0.003 mol) was reacted with monochloroacetic acid (0.284 g, 0.003 mol) in the presence of sodium hydroxide (10%) at ambient temperature. The mixture was stirred for 14 h. Then, the resulting mixture was neutralised with conc. HCl and the precipitate was filtered. 2, 5-dimethyl-4-methoxylbenzene thioacetic acid (**5**) formed was recrystallized with ethanol. White crystal, Yield: 20%, m. p. 84-86^oC. FTIR (KBr, cm^{-1}): 2922.2 (sp^3 C-H), 838.7 (Ar-C-H, bending vibration), 1602.8 (Ar-C=C-C), 1054 (C-O-C), 730.6 (C-S),

3377 (OH) and 1718.3 (C=O). δ_H ($CdCl_3$, δ -3.5, ppm): 6.92 (1H, s, Ar-H), 6.91 (2H, s, Ar-H), 3.78 (3H, s, $-OCH_3$), 2.34 (3H, s, Ar-CH_3), 2.04 (3H, s, Ar-CH_3) and 2.59 (2H, s, $-CH_2$).

2.6 Synthesis of 2, 5-dimethyl-4-methoxylbenzene acetylsulphide

Chloroform (20ml) was measured into conical flask, triethyl amine (1ml) was added and shaken. 2, 5-dimethyl-4-methoxylbenzene thiol (0.504 g, 0.003 mol) was dissolved in the mixture and acetylchloride (0.236 g, 0.003 mol) was added and stirred for 6 h at ambient temperature. Then, distilled water (100 ml) was added to the resulting mixture and extracted with chloroform (20 ml X 3). The extract was dried over magnesium sulphate and distilled. 2, 5-dimethyl-4-methoxylbenzene acetylsulphide (**6**) formed was purified by column chromatography (mobile phase: chloroform). Yellow liquid, Yield: 30% R_f: 0.87 ($C_6H_5CH_3$ Pet. ether and $CHCl_3$, 1:1:1), FTIR (KBr, cm^{-1}): 2922.2 (sp^3 C-H), 849.8 (Ar-C-H, bending vibration), 1051.1 (C-O-C), 715.6 (C-S) and 1707.1 (C=O). δ_H ($CdCl_3$, δ -3.5, ppm): 6.52 (1H, s, Ar-H), 7.05 (2H, s, Ar-H), 3.77 (3H, m, $-OCH_3$), 2.08 (3H, d, Ar-CH_3), 1.65 (3H, d, Ar-CH_3) and 3.38 (2H, m, $-CH_3$).

2.7 In Vitro Antibacterial Screening

The synthesized compounds were screened for antibacterial activities against ten bacteria, namely, *Xanthomonas phasecoli*, *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Pseudomonas solanacearium*, *Corynebacterium sepedonicum*, *Enterobacter aerogenes* *Xanthomonas vesicatorcia*, *Campylobacter sputorium*, *Klebsiellia aerogens* and *Serriatia rubidiae*. Agar well diffusion method as described by Murray *et al.* (1995) was used [13]. Streptomycin was used as a standard.

2.9 In Vitro Antifungal Screening

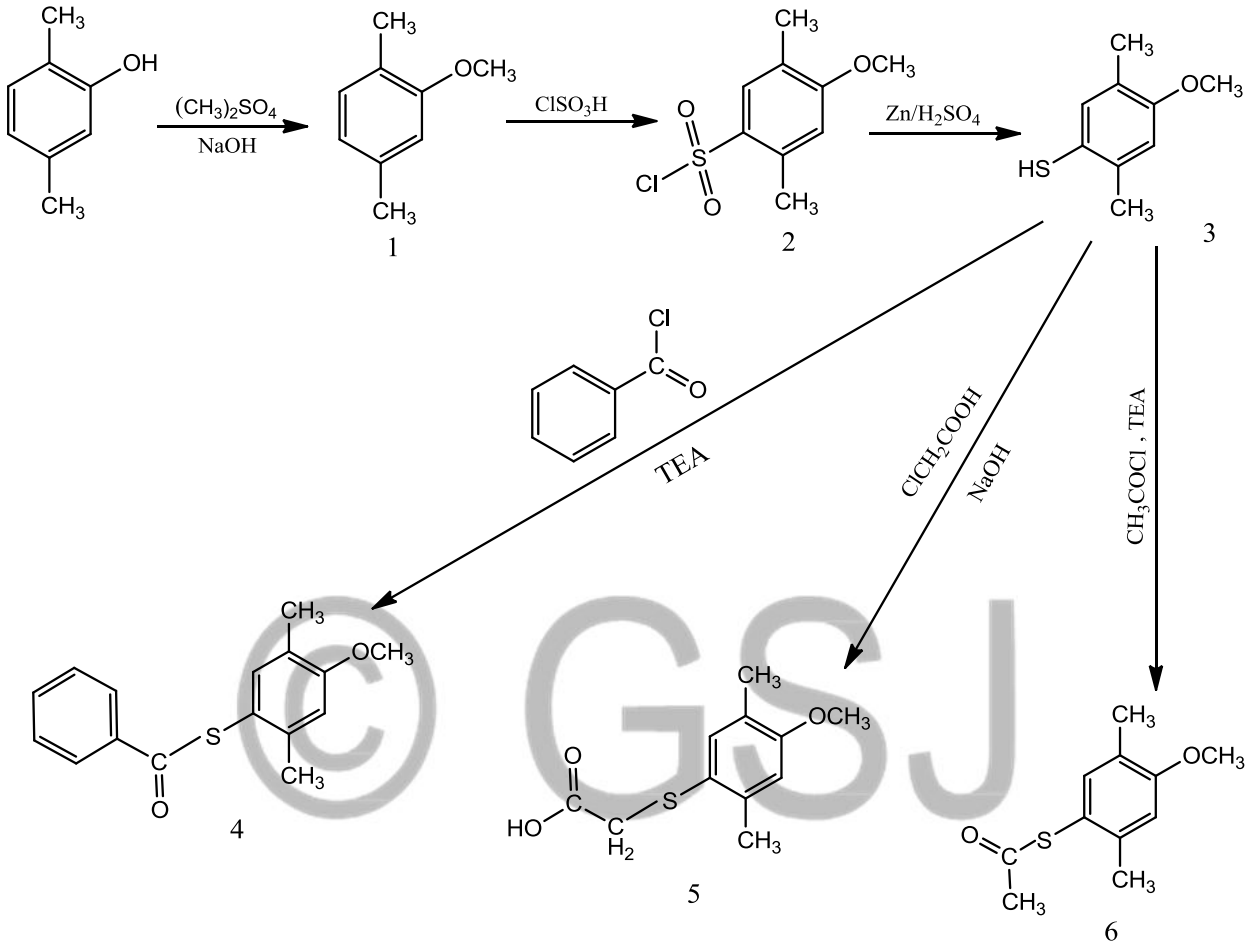
The synthesized compounds were screened for antifungal activities against three plant pathogenic fungi, namely, *Gloesporium piperatum, Collectotrichum trunartum, Cercospora sogina* using poisoned food technique as described by Murray *et al.* (1995) [9]. kocide was used as a standard at 0.25g/ml

3.0 RESULTS AND DISCUSSION

3.1 Synthesis and Characterization of Compound 1-6

The synthetic routes for compound **1-6** were depicted in scheme 1. Compounds **1** and **2** are commercially available. The synthesized compounds have similar properties. The FTIR spectra (Figure 1-4) for compound **3** to **6** showed aliphatic C-H stretching bands in the range of 2981.9 cm^{-1}-2922.2 cm^{-1}, the aromatic C-H stretching bands, aromatic C-H out-of-plane bending bands and aromatic C=C-C stretching bands were seen in the range of 3015.4 cm^{-1}-3000.4 cm^{-1}, 857.3 cm^{-1}-801.4 cm^{-1}, 1617.7 cm^{-1}-1599.0cm^{-1} respectively and vibrating bands for C-O-C occurred in the range of 1144.3 cm^{-1} -1051.1 cm^{-1}. All these bands confirmed the presence of 2, 5-dimethylanisole group in all the synthesized compounds. The band at 2728.4 cm^{-1} in Fig. 1 was characteristic band for SH bond and confirmed the presence of thiol in compound **3**. The stretching band for C-S bond was observed in all the spectra for compound **4**, **5** and **6** in the range of 738.0 cm^{-1}-715.6 cm^{-1}. The presence of carbonyl group in compound **4, 5** and **6** was established by bands (Fig. 2-4) at 1707.1 cm^{-1}, 1718.3 cm^{-1}, 1722.0 cm^{-1} respectively. Fig 3 revealed characteristic band for hydroxyl group at 3377.0 as present in compound **5**. The spectra of Proton Nuclear Magnetic Resonance from Figure 5 to Figure 8 also confirmed the structures of compounds **3** to **6.** The aromatic protons were observed between the range of 6.52 ppm and

7.20 ppm. The methyl protons were seen between 1.65 ppm and 3.79 ppm. The methylene protons were seen at 2.59 ppm. All these were characteristic peaks for the identified protons.



Scheme 1: Synthesis of Compound 1-6. **Compound 1:** 2, 5-dimethyl Anisole, **Compound 2:** 2, 5-dimethyl-4-methoxylbenzenesulphonyl chloride, **Compound 3:** 2, 5-dimethyl-4-methoxylbenzene thiol, **Compound 4:** 2, 5-dimethyl-4-methoxylbenzenebenzoyl sulphide, **Compound 5:** 2,5-dimethyl-4-methoxylbenzenethioacetic acid, **Compound 6:** 2, 5-dimethyl-4-methoxylbenzeneacetyl chloride.

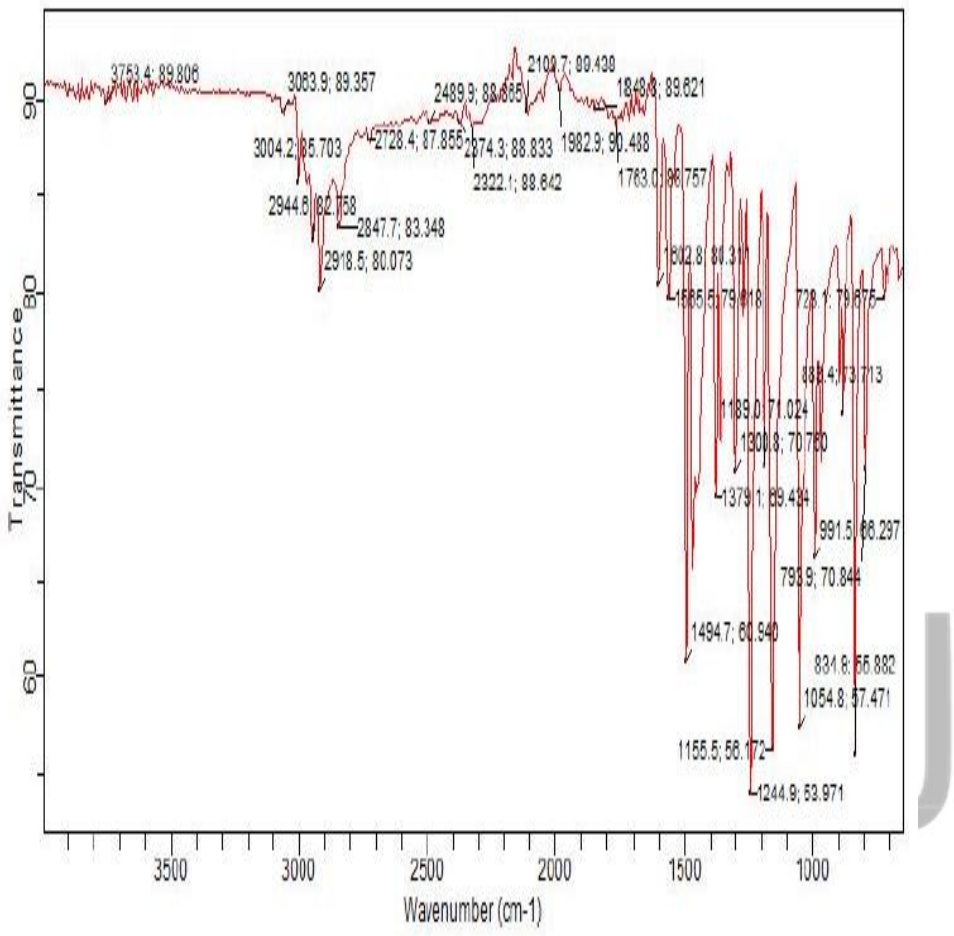


Figure 1: Fourier Transform Infra-Red Spectrum of 2,5-dimethyl-4-methoxylbenzene thiol

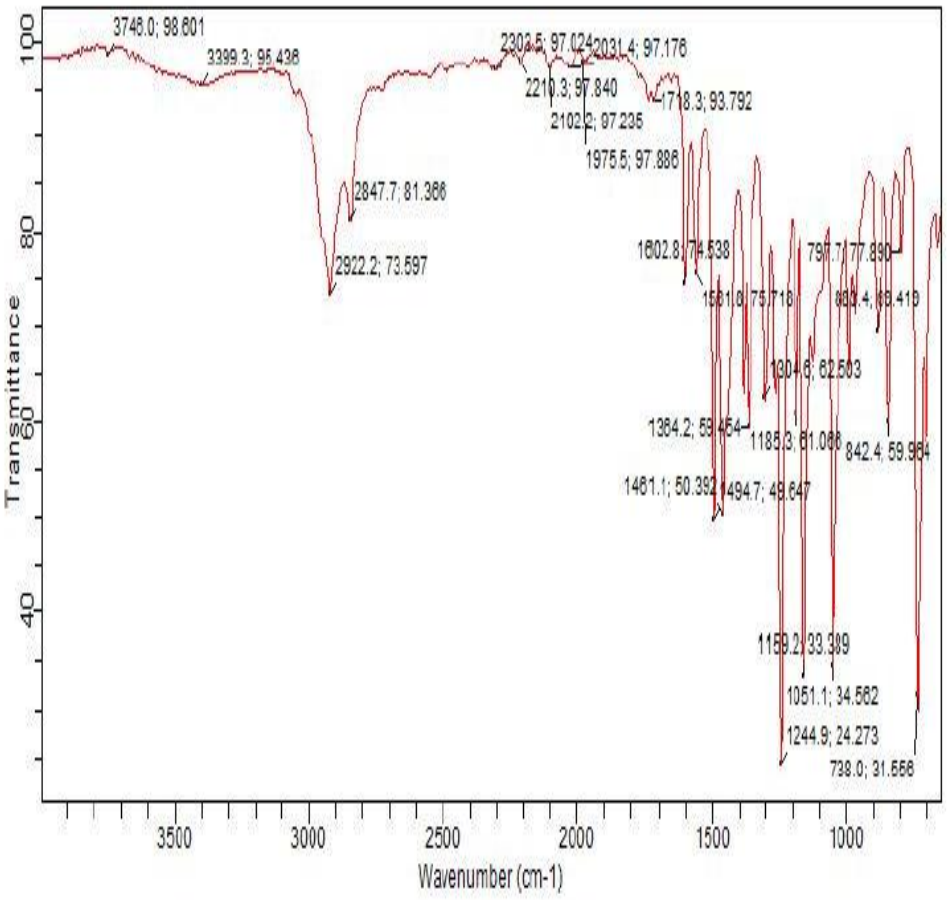


Figure 2: Fourier Transform Infra-Red Spectrum of 2, 5-dimethyl-4-methoxylbenzenebenzoyl sulphide

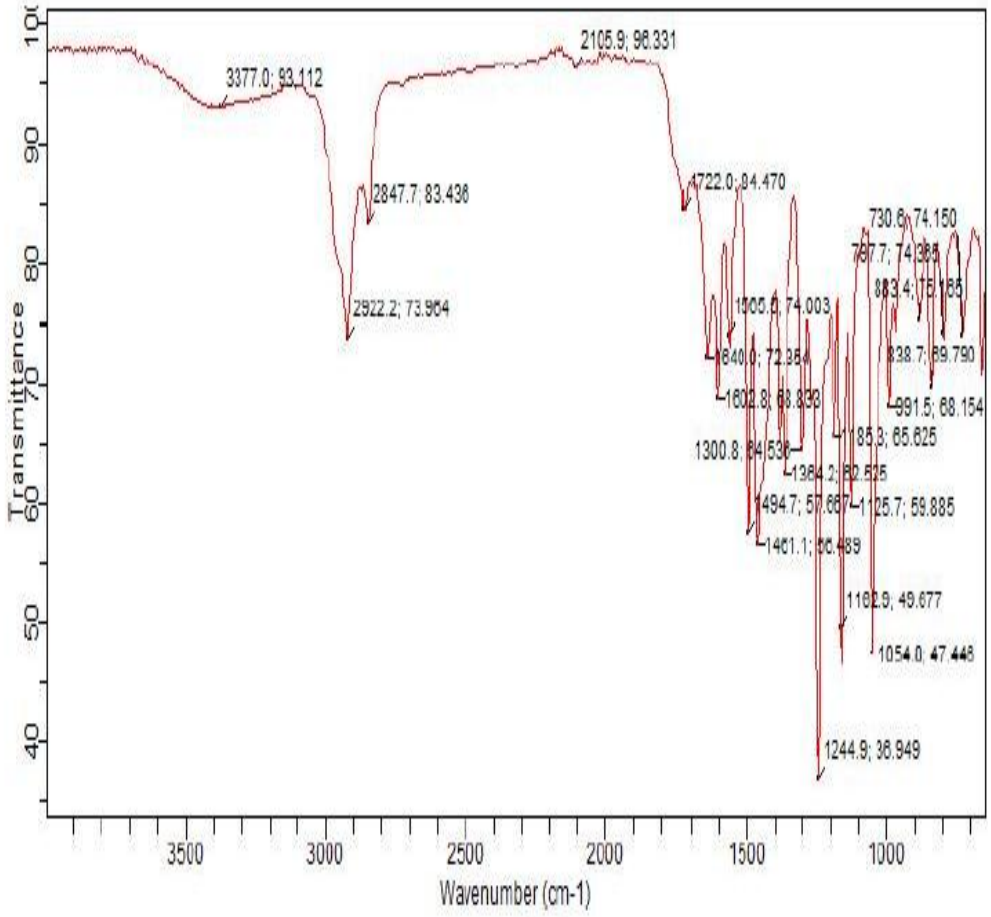


Figure 3: Fourier Transform Infra-Red Spectrum of 2, 5-dimethyl-4-methoxylbenzene thioacetic acid

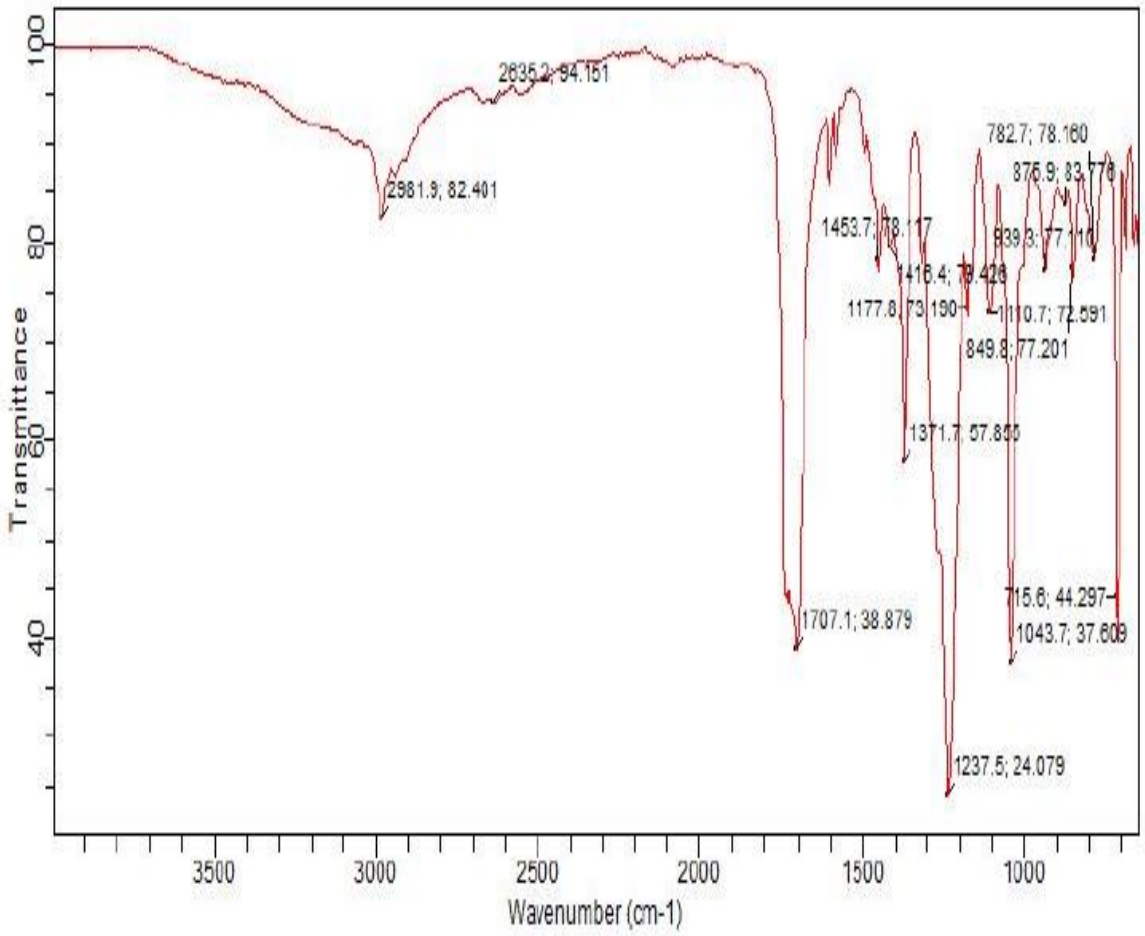


Figure 4: Fourier Transform Infra-Red Spectrum of 2, 5-dimethyl-4-methoxylbenzene acetylsulphide

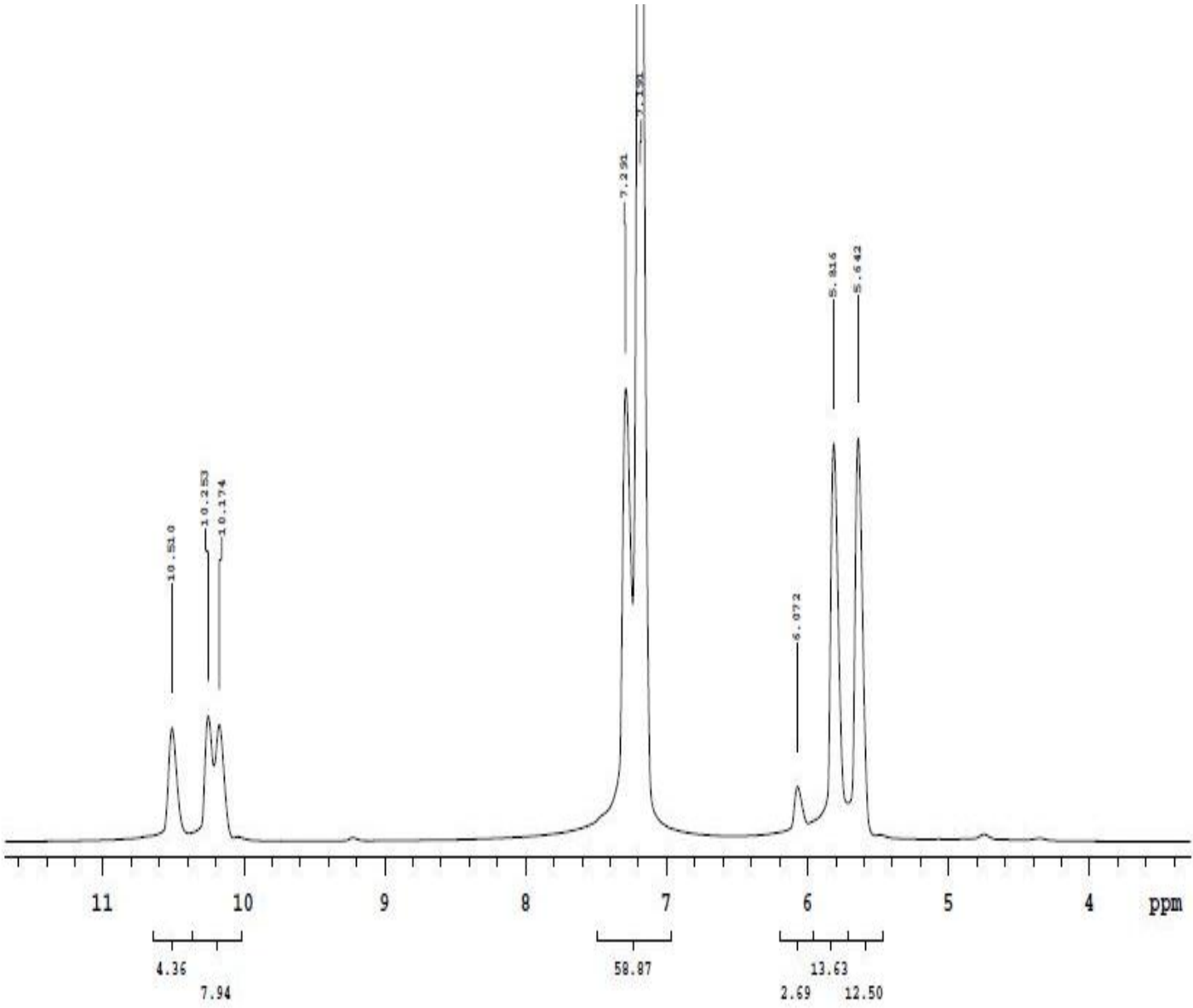


Figure 5: Proton Nuclear Magnetic Resonance Spectrum of 2,5-dimethyl-4-methoxylbenzene thiol

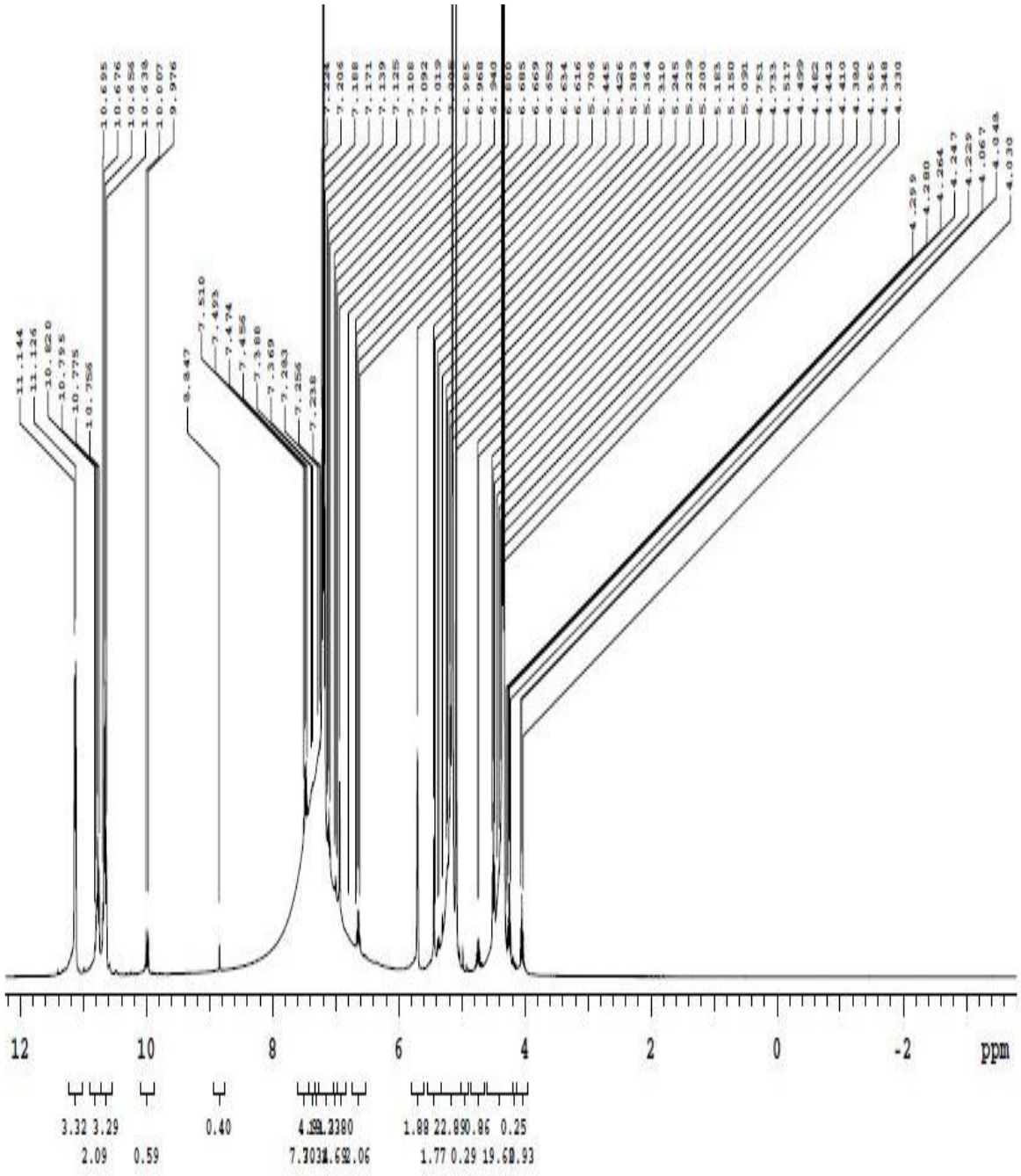


Figure 6: Proton Nuclear Magnetic Resonance Spectrum of 2, 5-dimethyl-4-methoxylbenzenebenzoyl sulphide

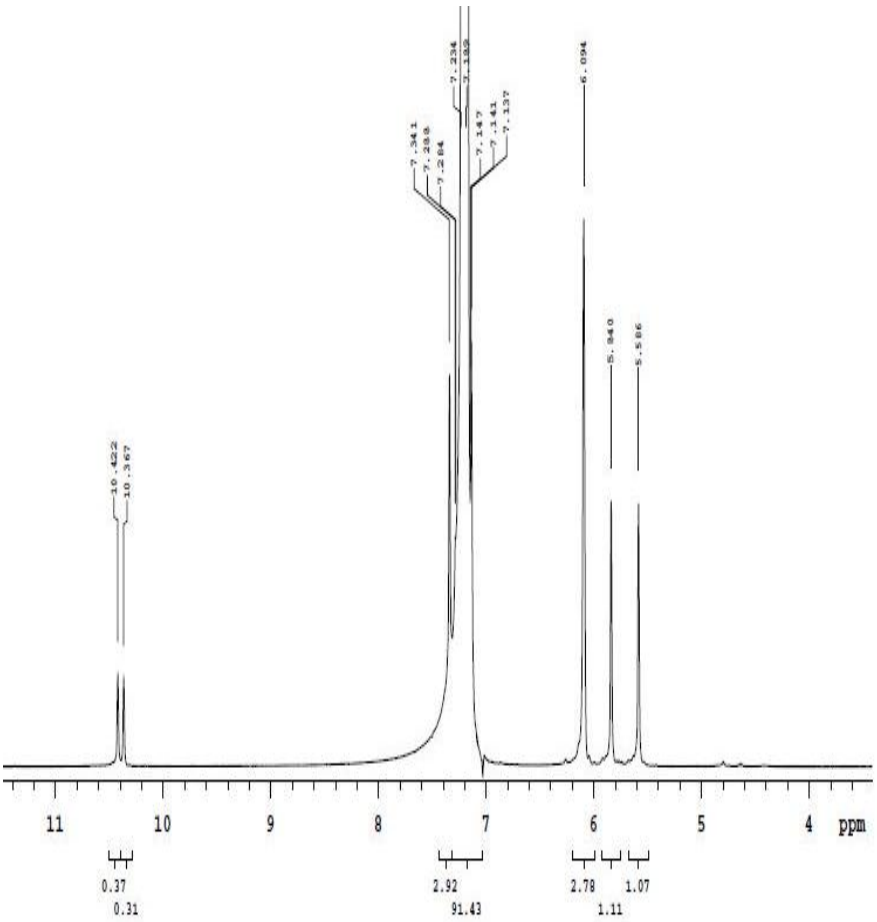


Figure 7: Proton Nuclear Magnetic Resonance Spectrum of 2, 5-dimethyl-4-methoxylbenzene thioacetic acid

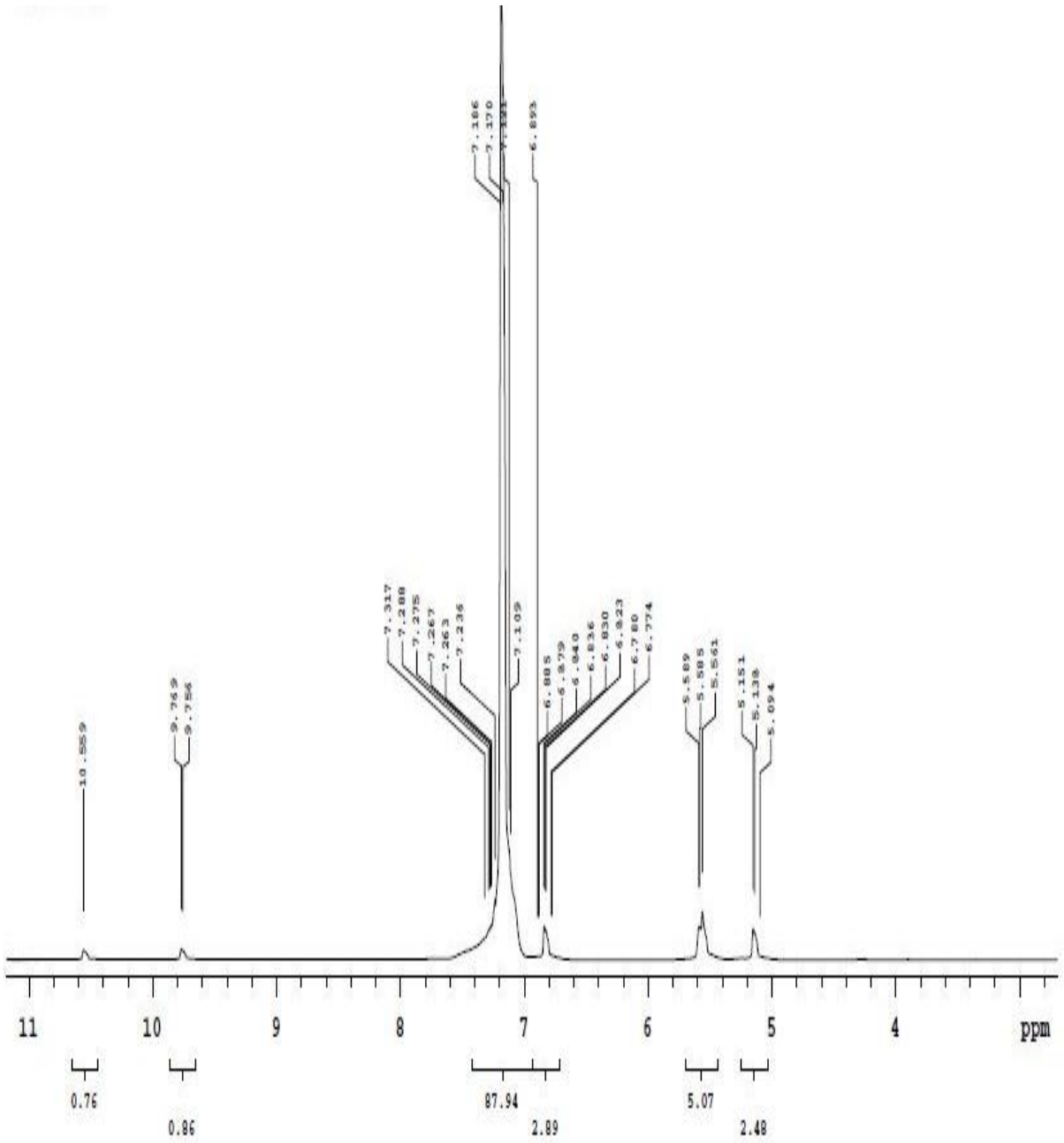


Figure 8: Proton Nuclear Magnetic Resonance Spectrum of 2, 5-dimethyl-4-methoxylbenzene acetylsulphide

3.2 In vitro Antibacterial Screening

The compound 4-6 were tested for their antibacterial activity against ten bacteria. namely, *Xanthomonas phasecoli*, *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Pseudomonas solanacearium*, *Corynebacterium sepedonicum*, *Enterobacter aerogenes Xanthomonas vesicatorcia*, *Campylobacter sputorium*, *Klebsiellia aerogens* and *Serriatia rubidiae* using streptomycin as a standard drug. The inhibitory results were reported in Table 1. It could be deduced from the table 1 that the compounds possessed some antibacterial properties, even though Streptomycin sulphate (standard) performed better than the tested compounds. The activities of compound 4 and 6 were stronger against *Xanthomonas vesicatorcia* and *Agrobacterium tumefaciens.* Compound 5 antibacterial activity was very low against all tested bacteria. Compound (6) showed a more useful antibacterial activity against all bacteria.

Table 1 : Antibacterial Activity of the Synthesized Compounds

Bacteria	Compound 4 (mm)	Compound 5 (mm)	Compound 6 (mm)	Streptomycin sulphate (mm)
Xanthomonas phasecoli,	3.50	3.00	3.00	20.00
Agrobacterium tumefaciens	10.50	6.00	9.00	22.00
Erwinia carotovora	7.00	1.00	7.00	21.00
Pseudomonas solanacearium	5.00	5.00	11.00	25.00
Corynebacterium sepedonicum	7.00	5.50	6.00	25.00
Enterobacter aerogenes	5.00	2.00	8.00	26.00
Xanthomonas vesicatorcia	11.00	3.50	11.00	27.00
Campylobacter sputorium	8.50	7.00	6.00	25.00
Klebsiellia aerogens	3.00	3.50	6.00	28.00
Serriatia rubidiae	2.00	1.00	3.00	20.00

Compound 4: 2, 5-dimethyl-4-methoxylbenzenebenzoyl sulphide, **Compound 5:** 2,5-dimethyl-4-methoxylbenzenethioacetic acid, **Compound 6:** 2, 5-dimethyl-4-methoxylbenzeneacetyl chloride. Streptomycin sulphate: Standard

3.3 In vitro Antifungal Screening

Compound **4** to **6** were screened for their antifungal activity against three plant pathogenic fungi, *Gloesporium piperatum, Collectotrichum trunartu* and *Cercospora sogina*, Kocide was used as

standard drug and ethyl acetate and chloroform were used as negative controls. The results of the antifungal activity were shown on Table 2. The standard exhibited stronger activity against all the tested compound. However, compound 4 demonstrated higher inhibition than the other compounds. Compound **5** has no inhibition against *Cercospora sogina*. Compound **4** inhibited growth of *Gloesporium piperatum* and *Cercospora sogina* better than compound **6**. Compound **5** exhibited antifungal activity against *Gloesporium* piperatum and *Collectotrichum trunartum*.

Table 2 Antifungal Activity of the Synthesized Compounds against Pathogenic Fungi

Fungi	Compound 4 (%)	Compound 5 (%)	Compound 6 (%)	Kocide (%)
Gloesporium piperatum	57.61	11.96	3.26	73.90
Collectotrichum trunartu	48.7	21.95	15.85	80.49
Cercospora sogina	55.68	0.00	27.27	84.00

Kocide: Standard Antifungi agent

4.0 Conclusion

2, 5-dimethyl Anisole (**1**), 2, 5-dimethyl-4-methoxylbenzenesulphonyl chloride (**2**), 2, 5-dim ethyl-4-methoxylbenzene thiol (**3**) , 2, 5-dimethyl-4-methoxylbenzenebenzoyl sulphide (4), 2 , 5-dimethyl-4-methoxylbenzenethioacetic acid (**5**), 2, 5-dimethyl-4-methoxylbenzeneacetyl chloride (**6**) were successfully synthesized. Compound **1** and **2** are commercially available w hile other synthesized compounds are not. The percentage yield of compound **3** to **6** was gene rally low. However, another synthetic methods may increase their yield. All the tested comp ounds exhibited antibacterial activity. Compound 6 showed a better result and stand a chance

of being a potential antibacterial agent. The results of antifungal activity revealed that all the tested compounds possessed antifungal properties. Compound 4 might be a useful antifungal agent against selected pathogenic fungi.

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