# Synthesis of New Type of Sugar Ligands Starting from D-Glucose And Screening Their Biological Activity

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## Abstract

This work includes two parts; the first one is the synthesis of two ligands starting from D-glucose, reaction of glucose with acetone in the presence of iodine afforded 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose(1), when (1) reacted with benzoyl chloride in pyridine gave 1,2:5,6-di-O-isopropylidene-3-*O*-(benzoyl)-α-D-glucofuranose (2), selective deprotection of (2) in 80% acetic acid yielded 1,2-*O*-isopropylidene-3-*O*-(benzoyl)--α-D-glucofuranose (3), the cleavage oxidation of (3) using sodium periodate gave the aldehyde (4), the reaction of (4) with 4-methylthiosemicarbazide and o-aminophenol afforded the sugar ligands (5) and (6) respectively.

The second part of this work contained the biological screening of the prepared sugar ligands against Gram + ve bacteria ***Staphylococcus Aureus*** (ATCC 25923), Gram – ve bacteria ***Escherischia Coli*** (ATCC 225922), ***Pseudomonas Aaruginosa*** (ATCC 27853), and against fungi ***Candida Albicans*** (ATCC 10231), this part of our study showed that only compound (5) exhibited activities against + ve bacteria ***Staphylococcus Aureus*** and Gram – ve bacteria ***Escherischia Coli*** at concentration of 5 ppm.

**الخلاصة**

يتضمن العمل الحالي قسمين أساسيين: القسم الأول تحضير المخلبيات السكرية أبتداءً من سكر د-كلوكوز وكما يلي: تم مفاعلة سكر د-كلوكوز مع الأسيتون وبوجود اليود كعامل مساعد واعطت هذه العملية المركب(1), عومل المركب (1) مع كلوريد البنزويل بوجود البريدين لينتج المركب (2), أزالة الحماية الأنتقائية للمركب (2) بواسطة استخدام 80% حامض الخليك أعطت المركب (3), تفاعل الأكسدة-أنشطار للمركب (3) باستخدام بيرأيودات الصوديوم أنتج الألديهايد (4), مفاعلة الألديهايد (4) مع 4-مثيل ثايوسيميكاربازايد و أوروثو-أمينوفينول انتجت المخلبيات السكرية (5) و (6) على التوالي.

بينما تضمن القسم الثاني من هذا العمل قياس الفعالية الحيوية للمخلبيات المحضرة تجاه Gram + ve bacteria ***Staphylococcus Aureus*** (ATCC 25923), Gram – ve bacteria ***Escherischia Coli*** (ATCC 225922), ,***Pseudomonas Aaruginosa*** (ATCC 27853), and against fungi ***Candida Albicans*** (ATCC 10231) حيث أظهر المركب (5) فقط وعند تركيز 5 جزء بالمليون فعالية واضحة تجاه + ve bacteria ***Staphylococcus Aureus*** و – ve bacteria ***Escherischia Coli***.

## Introduction

Carbohydrates are the most abundant biogenic class of compounds involved in a wide range of functions in living organisms. In case of cellulose, sugars act as scaffolding material in plants. Monosaccharide fragments are participating in glycolipids and glycoproteins and play an important role in various biological processes e.g. as building blocks for nucleotides and of the ADP/ATP energy-storage system.[1] Many sugar-metabolizing enzymes have been revealed to function with alkaline earth and transition metal ions in the active sites.[2–4]

Although the importance of sugar–metal interaction is known for many years, the field of sugar–metal complexes is still largely unexplored. Only for the past two decades, sugars received a growing interest as ligand components in bioorganic chemistry due to their enantiomerically pure

natural abundance and their polyfunctionality. The vicinal functional groups feature many donor atoms forming stable chelate complexes.[5–9] Furthermore, carbohydrates combine interesting properties like stable chiral scaffold and supramolecular arrangement. Well-known modification strategies for introducing additional donor groups into the sugar backbone are *N*-glycosylation with polyamines and nucleophilic substitution. Of bromoethyl-*O*-glycosides.[10-13]

Azomethines constitute a densely populated class of compound readily available by condensation of a carbonyl compound with an ammonia derivative.[14–15] Their widespread application in organic synthesis is based on the sensitivity of the C=N double bond towards attacks by nucleophiles and radicals, and on various possibilities offered by substituents on the nitrogen especially when they are of heteroatomic nature.[16]

## *Experimental Section*

## Part I: Synthesis

Aldrich, Fluka AG, BDH, Riedel-de Haen AG, Acros Organics, Janssen, Hopkin & Williams and Ajax, supplied all chemicals used.

***Synthesis of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose(1)***

D-glucose (1.00 g, 5.55 mmol) was added to a solution of iodine (0.30 g, 1.18 mmol) in acetone (50 mL), the suspension was stirred at room temperature for 4 h. After this time the sugar had dissolved completely. The reaction was quenched by the addition of dilute sodium thiosulfate solution to render the reaction mixture colorless, the acetone was removed in vacuum. The aqueous solution was transferred to a separatory funnel and extracted with chloroform (3 x 20 mL), the combined organic layers were washed with distilled water (100 mL), dry ($Na_2SO_4$) and solvents removed in vacuum to afford the crude product. Recrystallization (diethyl ether/petrol) afforded di-acetone glucose(1) as colorless crystals (1.09 g, 80%). m.p. 109–110 $^0$C.

***Synthesis of 1,2:5,6-di-O-isopropylidene-3-O-(benzoyl)-α-D-glucofuranose(2)***

To a stirred solution of the glucofuranose(1) (1.7 g) in pyridine (4.6 mL) benzoyl chloride (1 mL) was added dropwise at -10 $^0$C. Stirring was continued at this temperature for 4 h, the pink solution was poured into (10 mL) of water, and this mixture was diluted with (60 mL) of water. The precipitating oil, which solidifies to a solid mass, was filtered off, washed with water and recrystallized from 70% aqueous methanol. Yielded (2) (0.196 g, 88%), m.p. (83-84 $^0$C).

***Synthesis of 1,2-O-isopropylidene-3-O-(benzoyl)—α-Dglucofuranose(3)***

A solution of compound **(2)** (3.64 g,10 mmol) in 80% acetic acid (50 mL) was kept at room temp. for (48 h.), after which time the solution was concentrated and co-evaporated with n-butanol (3x15 mL), the residue was extracted with ethyl acetate then dried, evaporation of solvent followed by flash chromatography afforded the product **(3)** as a white amorphous material (2.95 g, 91% yield), m.p. (75-77$^o$ C).

***Synthesis of Aldehyde(4)***

A solution of diol (3) (1 mmol) in a small amount of ethanol was added over (30min.) to the solution sodium periodate (1.5 mmol) in water (10 mL), the oxidation was allowed to proceed for (1 h) at (0$^o$ C), the solvents were removed, the residue was taken up in ethyl acetate (20 mL) and washed with brine, water and dried with $MgSO_4$, evaporation of solvent under reduced pressure followed by column chromatography of the residue using (EtOAc: light petroleum 2:1) as eluent afforded the pure aldehyde (4) as a colorless syrup (82%).

***Synthesis of Schiff bases (5 and 6)***

A solution of amine (1.2 mmol) in a small amount of ethanol was added to the solution of aldehyde (4) (1 mmol) in (25 mL) absolute ethanol, the solution was refluxed for (30 min.), the

solvent was evaporated under reduced pressure to give the compounds: (5) as a light yellow solid m.p. (102-104$^{o}$C) and (6) as a yellow solid m.p. ( 122-125$^{o}$C).

***Part II: Biological Screening; Antimicrobial Susceptibility Tests***

The biological activity of some prepared compounds was tested against strains or Gram + ve bacteria ***Staphylococcus Aureus*** (ATCC 25923), Gram – ve bacteria ***Escherischia Coli*** (ATCC 225922), ***Pseudomonas Aaruginosa*** (ATCC 27853), and against fungi ***Candida Albicans*** (ATCC 10231).

All procedures were conducted under sterilized conditions at the Department of Biology, University of Kerbala; Antimicrobial activity was carried out by agar diffusion method.

Kirby-Bauer [16] method was used to determine the antimicrobial activity for two prepared compounds (**5 and 6**); ampicillin was used as a control.

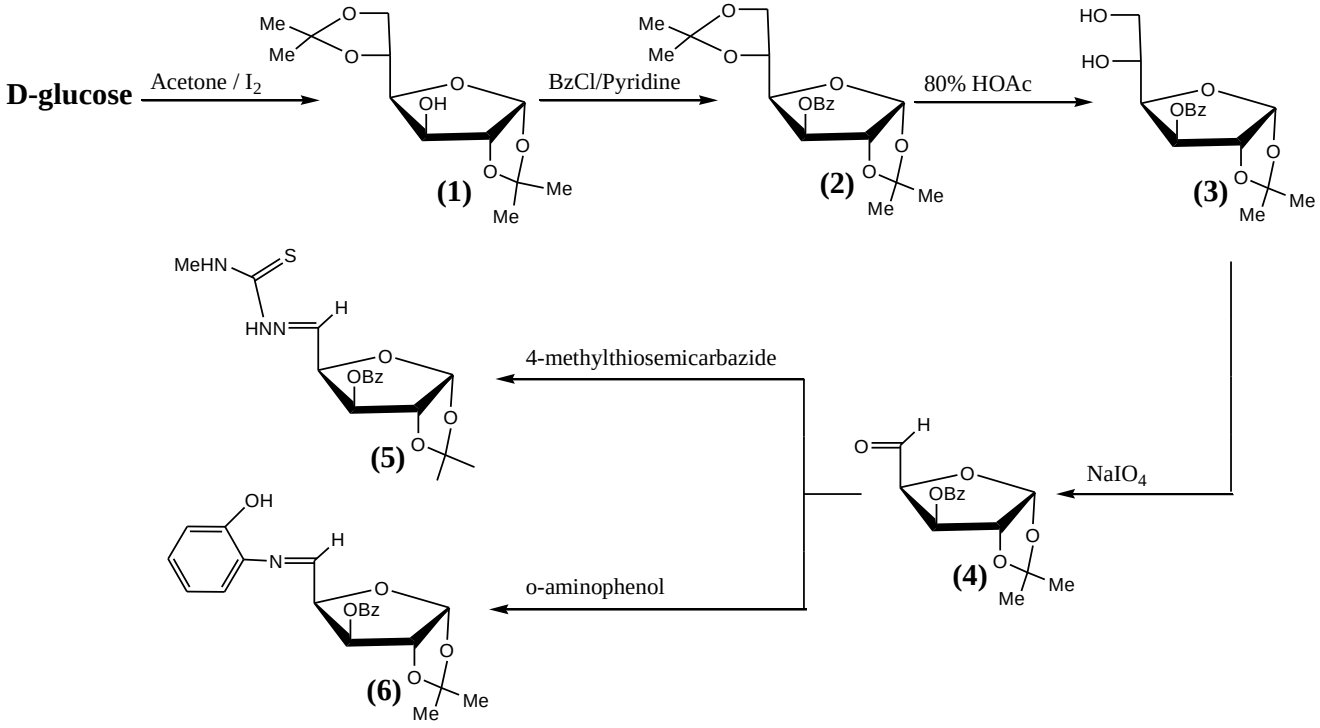
***Procedure*** [17]***:***

1.Five concentrations were prepared from each of the above products (1-5 ppm).
2.DMSO was used as a solvent.
3.Microbial broth was prepared according McForland technique .
4.(0.01 mL) of loopfull of microbial broth was seeded on a plate of Muller-Hinton agar.
5.Each plate contained one type of microbial tested against product and control.
6.These plates were incubated at 37$^{o}$ C for 24 h.
7.The diameters of the inhibition zone of each compound were measured.

## *Results and Discussion*

***Part I: Synthesis***

The overall synthetic strategy of this work is shown in the following scheme:




**Scheme (1) The overall synthetic routes**

The reaction of D-glucose with acetone in the presence of iodine afforded compound (1). Figure (1) shows the FT-IR spectrum of (1); the band at 3425 cm$^{-1}$ refers to the stretching of the hydroxyl group at C-3, the bands at 2963 cm$^{-1}$ and 2926 cm$^{-1}$ are attributed to the asymmetric and symmetric stretching of methyl and metyhlene groups. The bending vibrations bands of the methyl

and methylene groups lie at 1440 $cm^{-1}$ and 1375 $cm^{-1}$, the stretching band of (C-O) groups centered around 1075 $cm^{-1}$ .

Esterification of compound (1) with benzoyl chloride in pyridine as a solvent gave compound (2). FT-IR spectrum of (2) shown in figure (2); the disappearance of the (O-H) stretching band around 3425 $cm^{-1}$ in addition to the appearance of new bands at: 3050 $cm^{-1}$ for aromatic (C-H) stretching, 1727 $cm^{-1}$ for benzoate (C=O) stretching, 1600 $cm^{-1}$ and 1500 $cm^{-1}$ for aromatic (C=C) stretching and 900-650 $cm^{-1}$ for aromatic (C-H) bending out of plane is an excellent evidence for formation of (2).

Treatment of compound (2) with 80% acetic acid selectively removed the isopropylidene at the positions 5 and 6 of the sugar molecule (3).

FT-IR spectrum of (3) figure (3) showed the following bands; 3425 $cm^{-1}$ and 3300 $cm^{-1}$ for hydroxyl stretching groups which introduced a very good evidence for formation of (3).

The reaction of compound (3) with sodium periodate in mixture of alcohol-water gave the aldehyde (4).

FT-IR spectrum of (4) figure (4) showed the bands at: 3070 $cm^{-1}$ for aromatic (C-H) stretching, 2987 $cm^{-1}$ and 2888 $cm^{-1}$ aliphatic (C-H) stretching, 2800 $cm^{-1}$ and 2700 $cm^{-1}$ aldhehydic (C-H) stretching, 1725 $cm^{-1}$ aldehydic (C=O) stretching and 1600 $cm^{-1}$ and 1500 $cm^{-1}$ aromatic (C-C) stretching.

Sugar ligands (5) and (6) were formed when compound (4) was treated with 4-methylthiosemicarbazide and o-aminophenol in ethanol. The ligand (5) classified as (N,S) ligand while ligand (6) classified as (N,O) one.

FT-IR spectrum of (5) figure (5) showed the following bands: 3250 $cm^{-1}$ secondary amine (N-H) stretching, 2965 $cm^{-1}$ and 2887 $cm^{-1}$ aliphatic (C-H)stretching, 1645 $cm^{-1}$ imine (C=N) stretching, the mentioned bands introduce very good evidence of the formation of (5).

While FT-IR spectrum of (6) figure (6) showed approximately the same bands except the band at 3350 $cm^{-1}$ which refers to the phenolic (O-H) stretching.

***Part II: Biological Screening***

The sugar ligand (5) exhibited a biological activity against + ve bacteria ***Staphylococcus Aureus*** (ATCC 25923), Gram – ve bacteria ***Escherischia Coli*** (ATCC 225922) at the concentration of 5 ppm, this may be attributed to the presence of the (C=S) group in the ligand (5) which affect the metabolism of bacteria[(1)].

**Table (1) Antimicrobial activities of compounds (5 and 6) (5µg.$mL^{-1}$)**

| Compound | *E. coli* | *S. aureus* | *P. aeruginosa* | *C. albicans* |
|---|---|---|---|---|
| DMSO | — | — | — | — |
| Ampicillin | 10 | 27 | 30 | — |
| **5** | 4 | 5 | — | — |
| **6** | - | - | - | — |

- Zone of inhibition in mm.

***Acknowledgment***

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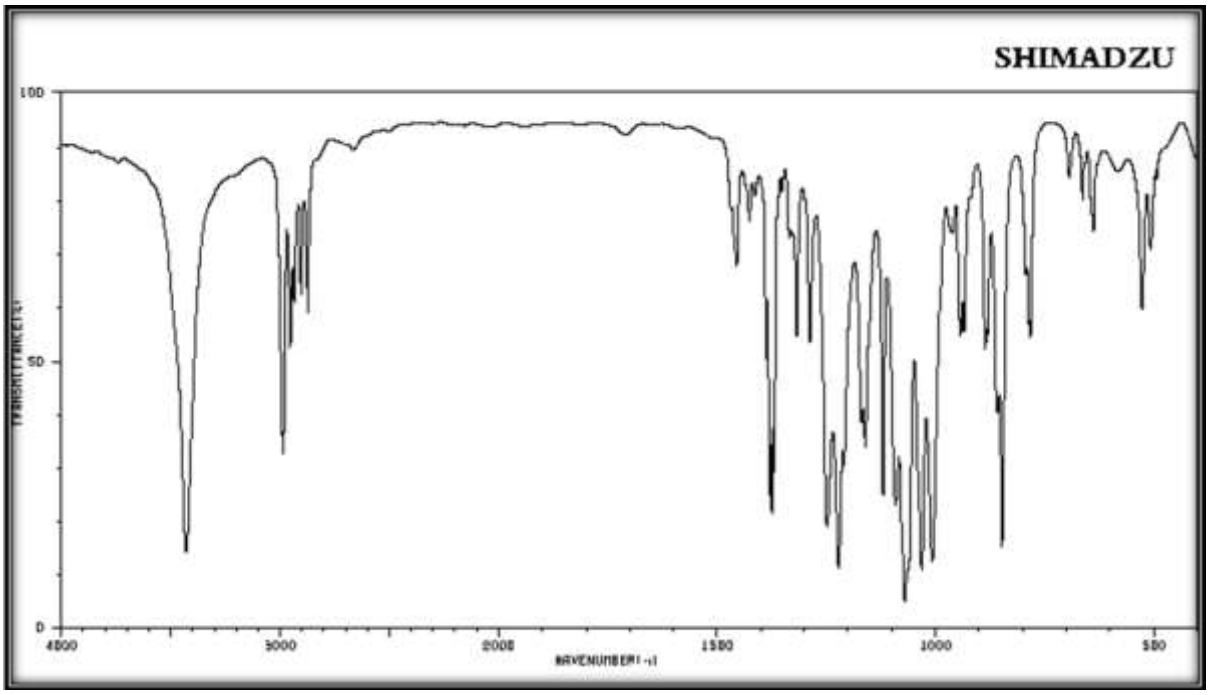


**Figure (1) FT-IR spectrum of compound (1)**

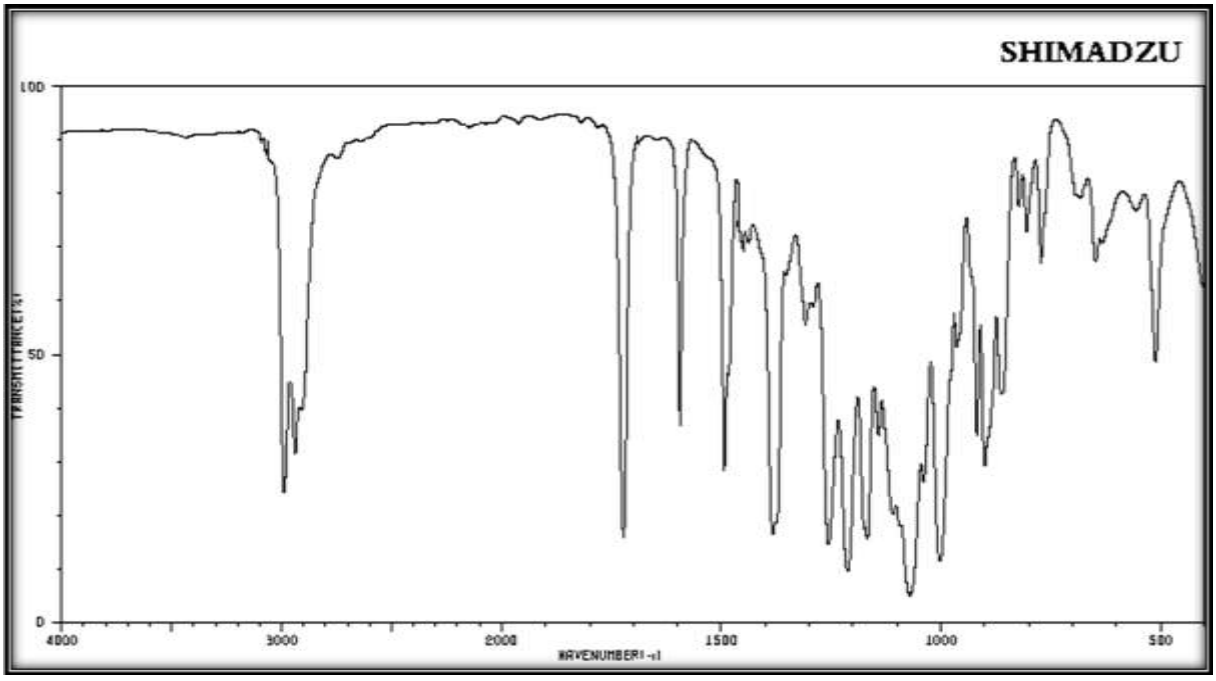


**Figure (2) FT-IR spectrum of compound (2)**

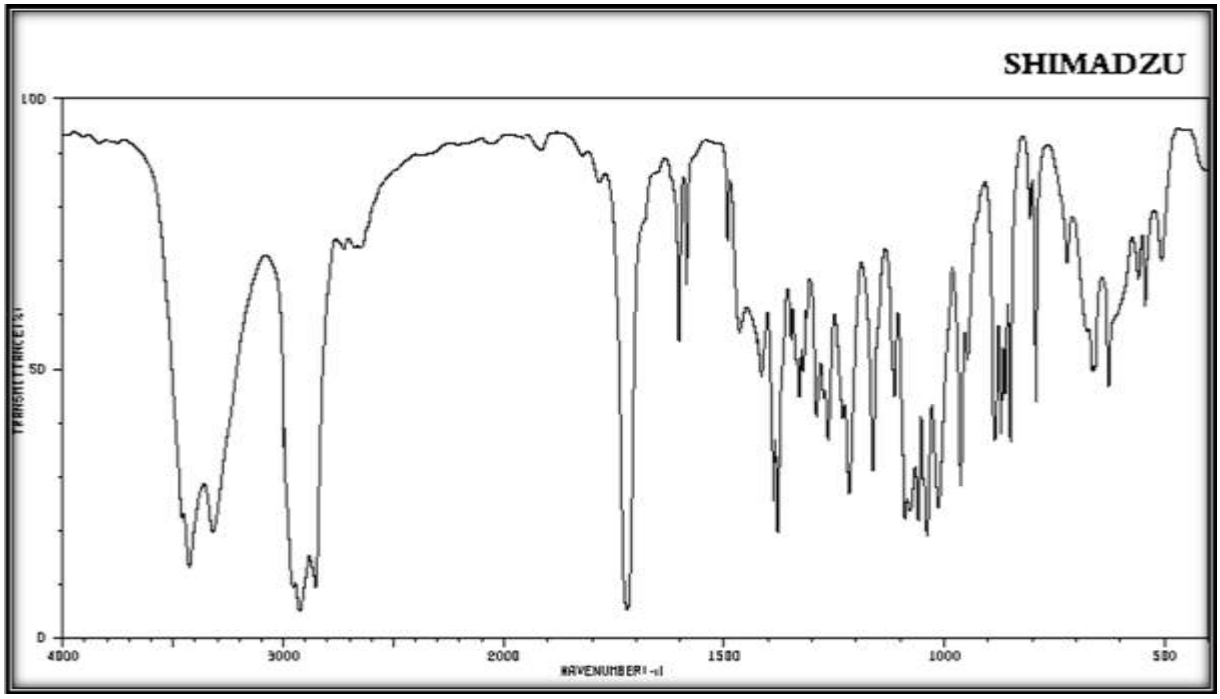


**Figure (3) FT-IR spectrum of compound (3)**

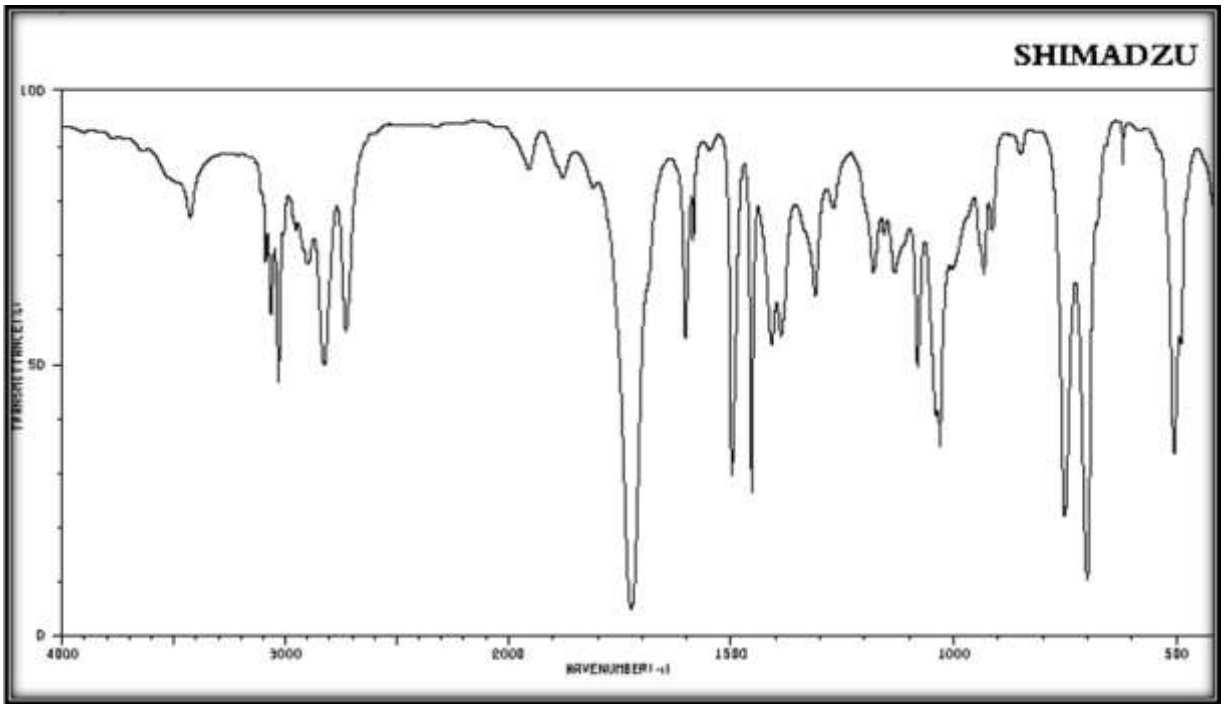


**Figure (4) FT-IR spectrum of compound (4)**

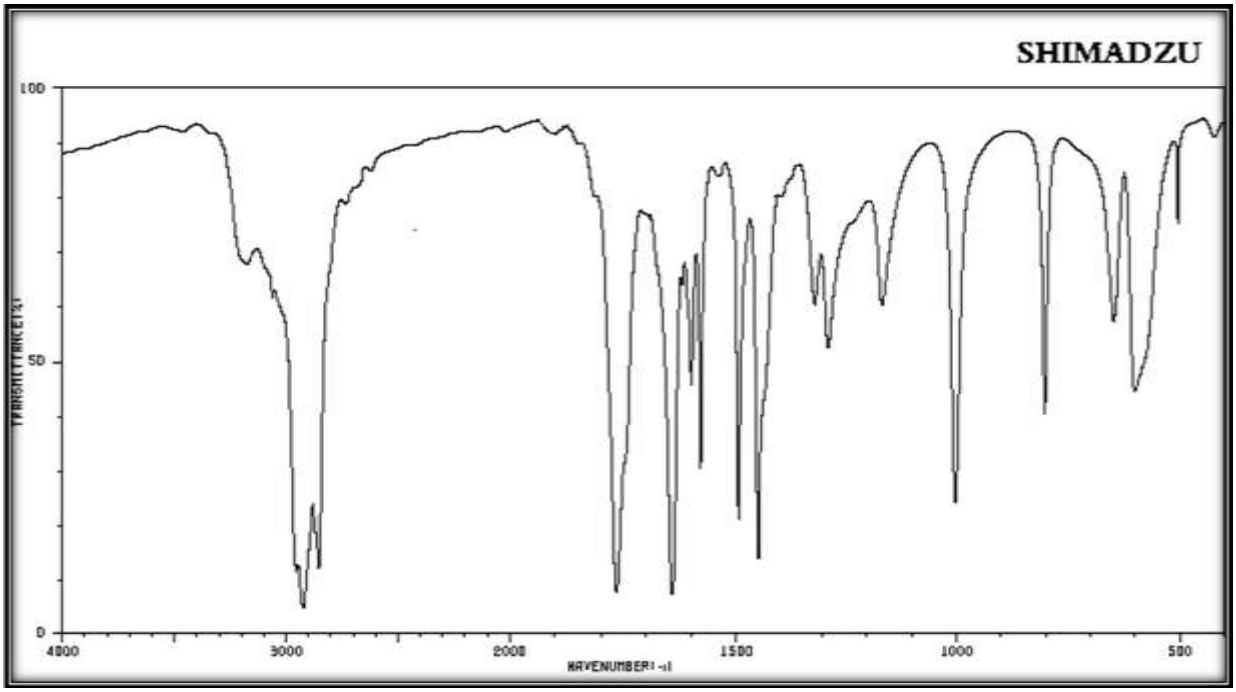


**Figure (5) FT-IR spectrum of compound (5)**

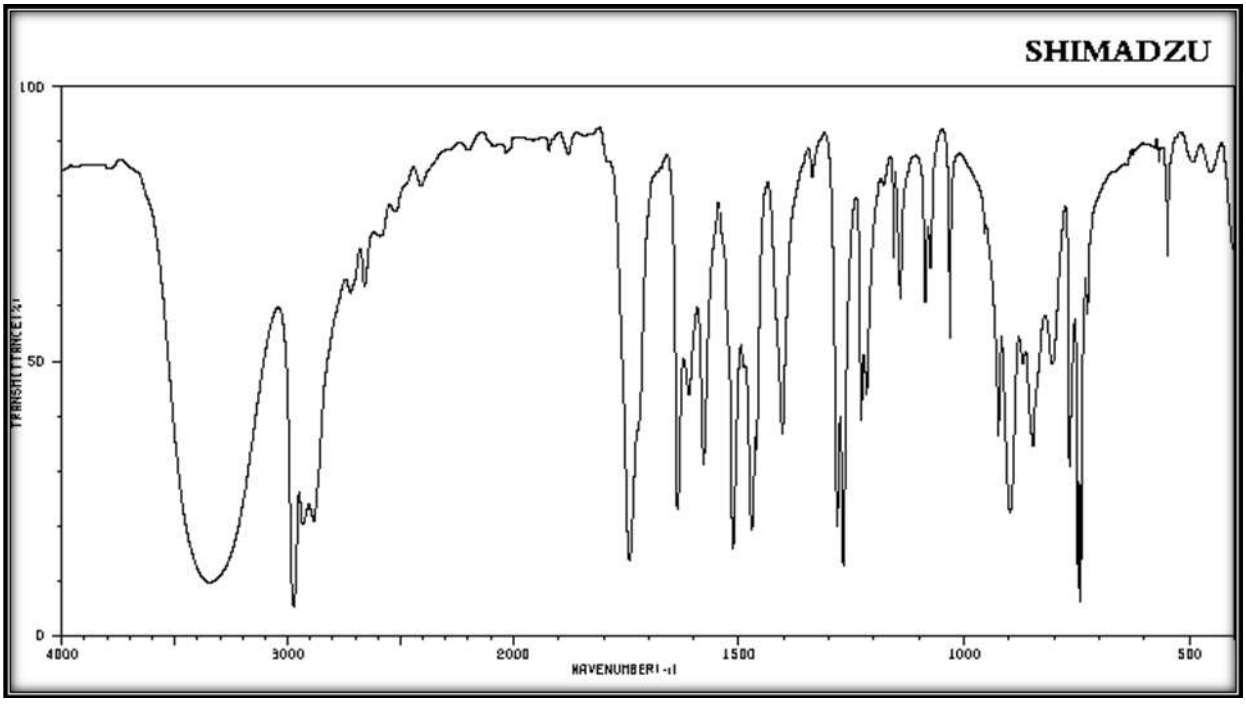


**Figure (6) FT-IR spectrum of compound (6)**