An-Najah National University

Faculty of Graduate Studies

Synthesis of Aromatic Amino-acid Esters from 2-Phenylaminethanol and Exploring some of their Biological Activities

By

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Dedication

To my parents for their help, care, and prayer for me.

To my husband Odai for his support, love, and encouragement.

To my sisters Rua, Selena, and Ibtehal for their support and concern

To all of my family members for their encouragement.

To my brothers Samer,Abdallah, Bara, Khalid, and Hamzah for their love, sincere feelings, and moral support.

To my friends for their continuous support.

To all who prayed for me.

To all whom I love and know.

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الإقرار

انا الموقعة ادناه مقدمة الرسالة التي تحمل العنوان:

Synthesis of Aromatic Amino-acid Esters from 2-Phenylaminethanol and Exploring some of their Biological Activities

أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الاشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو بحث علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية اخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's work and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالبة: أسيل ماهر حمامرة**

Signature: **التوقيع:**

Date: **التاريخ: 20/6/2021**

List of Contents

List of Tables

List of Figures

List of Schemes

List of Abbreviations

Symbol	Abbreviation
FT-IR	Fourier Transform Infrared
^{1}H-NMR	Proton Nuclear Magnetic Resonance
ROS	Reactive Oxygen Species
BHT	Butylated Hydroxyl Toluene
HPLC	High- Performance Liquid Chromatography
TLC	Thin Liquid Chromatography
UV	Ultra Violet
GC	Gas Chromatography
DPPH	α-Diphenyl-β-picrylhydrazyl
MIC	Minimum inhibitory concentration
PNPB	P-Nitrophenyl butyrate

Synthesis of Aromatic Amino-acid Esters from 2-Phenylaminethanol and Exploring some of their Biological Activities

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Abstract

Four compounds of aromatic amino-acid esters were prepared from the reaction of benzoic acid, and its derivatives (2-hdroxy, 3-hydroxy, 4-hydroxy benzoic acid with 2-phenyleaminethanol. The structures of these amino-acid esters were established by proton nuclear magnetic resonance (1H-NMR), Fourier transforms infrared (FT-IR), and carbon-13 (C13) nuclear magnetic resonance. The aromatic amino-acid esters were tested for their antioxidant, antibacterial, antifungal, anticancer, anti-lipase, and anti-α-glycosidase activities. The compounds' activity as antioxidants in DPPH was about (IC_{50} > 0.5 mg/ml), while the IC_{50} value of Trolox was 0.1 mg/ml. The compounds were also tested for their antibacterial and antifungal activities against*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), and *Candida albicans* and showed MIC value (4 mg\ml). The result didn't show significant activity toward cancer cells. Moreover, for lipase (IC_{50} = 231 mg/ml) and IC_{50} = 87.5 mg/ml for α-glycosidase inhibitory activity test, which is the same as acarbose. The synthesized aromatic compounds showed significant activities in most of the

tests. The best results were in DPPH antioxidant, antimicrobial, and α-glycosidase inhibitory activities.

The synthetic compounds were prepared efficiently by the reaction of acid esters and 2-phenylaminethanol. The compound's identity was confirmed using a spectroscopic method such as ^{1}HNmr, UV, carbon 13, and IR. The amino acid ester compounds were identified depending on some physical properties such as thin-layer chromatography and melting points. Many tests were used to confirm the compounds' biological activity for antioxidant, antibacterial, antifungal, anticancer, anti-lipase, and α-glycosidase activities. They showed significant activity in most of the tests. The best results were found in DPPH antioxidant, antimicrobial, and α-glycosidase. Overall, the results of these compounds support other researchers to complete studies for further clinical trials and make them as best choices of human drugs.

Chapter One

General Introduction

1.1 Amino acid esters

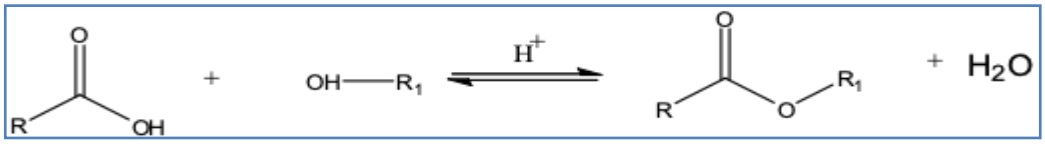
In organic chemistry, compounds that contain functional group of nitrogen atom with a lone pair are known as amines. They derivatives from ammonia, where at least one hydrogen atom has been supplanted by a substituent, such as an alkyl group are called alkyl amines, or aryl group are then called aryl amines.The most essential amines include amino acids, trimethylamine, biogenic amines and aniline (1, 2).

Acid ester as an organic compound can be produced from acid and alcohol reactions. Esters generally have a pretty smell, and they are considered high-quality solvents for broad groups of plasticizers, lacquers, plastics, and resins. They also contribute to biological activities, including antibacterial, antifungal, anticancer, anti-platelet, anti-inflammatory, and enzyme inhibitors (3, 4).

The amino acid is defined as any organic molecule that contains a primary amino group (-NH2), side chain (R group), and acidic carboxylic group (-COOH) (5). The main elements of an amino acid are carbon (C), oxygen (O), hydrogen (H), and nitrogen (N). There are around 500 amino acids discovered during the last 30 years. They can be classified according to pH, polarity, type of side-chain group [aliphatic, acyclic, aromatic containing

sulfur or hydroxyl, etc.]. Moreover, according to the location of the core structural-functional group [α, γ, β, δ] amino acid (6).

The most common esters are derived from carboxylic acid, with the formula RCOOR´. They are produced by the reaction of alcohols with carboxylic acids in sulfuric acid or hydrochloric acid. An O- alkyl group substitutes a place of at least one hydroxyl group (OH) in a process called esterification, as illustrated in scheme 1.1. Fischer esterification reaction is an equilibrium reaction, and it tends to be directed toward the product side by the use of an excess of one of the reactants or by removing water (7, 8).



S**cheme (1.1):** General equation of Fischer esterification.

1.2 Benzoic acid, phenolic acid.

The simplest aromatic carboxylic acid is Benzoic acid ($C_7H_6O_2$), colorless with molecular weight of 122.13. It has a benzene ring directly bonded to the carboxylic group. It is found naturally in animal tissues, plants, fermented products through microbial metabolism, and can be synthesized in laboratories. It is used as nucleating agent, additive, intermediate, catalyst, and/or stabilizer in coolant, solvent, plastic, photography, textiles, paper, dye industries, and pesticide. Moreover, it can be used as a flavoring and/or preservative agent in pharmaceutical products, food, hygiene, and cosmetics. Also, benzoic acid derivatives and related benzoic compounds, such as their

salt (calcium, potassium, and sodium benzoates), hydroxybenzoate esters, benzoyl peroxide, alkyl benzoate esters, ethyl p-hydroxybenzoate, Sodium ethyl hydroxybenzoate, benzaldehyde, and benzyl alcohol can be naturally present or industrially synthesized. They are commonly used in different Industrial fields as flavoring agents, antifungal, antibacterial preservatives in oral, topical, and parenteral drugs (9-11).

The activity and effectiveness of benzoic acid and its derivatives have been proved against yeasts and molds, including *Penicillium, Eurotium, Saccharomyces, Candida, debaryomyces, PichiaKloeckera, Aspergillus, Kluyveromyces, and* Zygosaccharomyces. It has also demonstrated against bacteria that belong to the species *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, Leuconostoc dextranicum, Leuconostoc mesenteroides, Lactobacillus plantarum, Lactobacillus brevis,* and *Pseudomonas aeruginosa* (9, 12).

Acid esters and phenolic compounds include a broad set of molecules that possess polyphenol structure, which means several hydroxyl groups attach to aromatic rings. Phenolic compounds and acid esters are widely used in various sectors as antimicrobial preservatives in food, cosmetics, and pharmaceutical preparations. Furthermore, these compounds' biological effect has been observed such as antibacterial, anti-inflammatory, antioxidant, antiviral, anti-carcinogenic, and anti-atherogenic properties (13). One of the basic classes of phenolic compounds is phenolic acids or phenolic carboxylic acids, which have high antioxidant activity and other applications, as shown in figure 1.1 (14).

Phenolic acids can be classified into hydroxycinnamic acids and hydroxybenzoic acids groups. Syringic, p-hydroxybenzoic, vanillic, and protocatechuic acids are the most common hydroxybenzoic acids derived from benzoic acid. On the other hand, caffeic, ferulic, sinapic, and p-coumaric acids are the most prevalent hydroxycinnamic acids produced from cinnamic acid (14, 15).

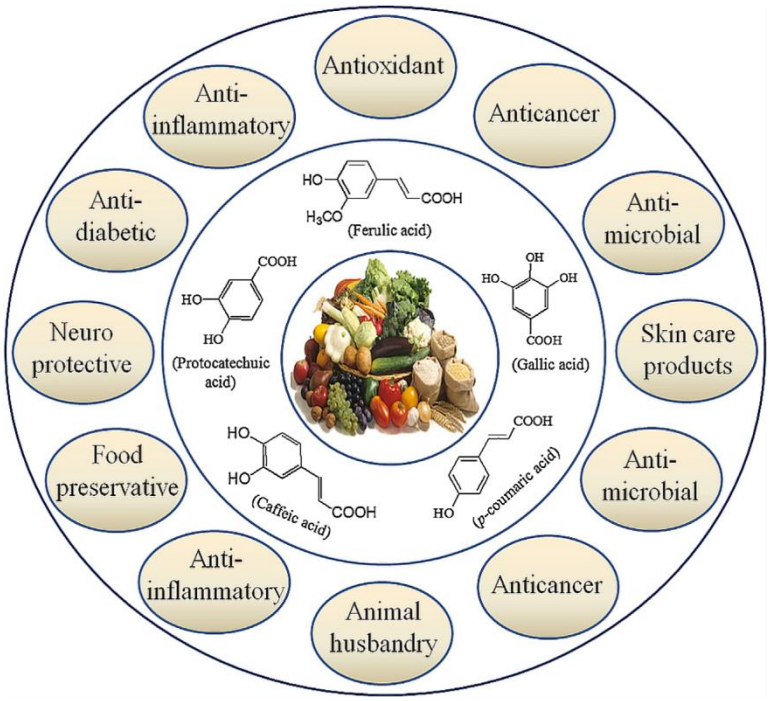


Figure (1.1): Applications of phenolic acids (16)

The presence of reactive oxygen species (ROS) like hydroxyl (OH), peroxyl (RO_2), and superoxide (O_2) in the body lead to degradation in tissues and DNA damage, thus contribute to the occurrence of cancer and other diseases (15). For this reason, many types of research have focused on the discovery and synthesis of antioxidant compounds to provide protection against oxidative stress. Phenolic acids have high antioxidant activity because of the high reactivity of phenol moiety from hydroxyl substituent on the aromatic

ring (9). The antioxidant activity of the phenolic compounds and phenolic acids depends on their structure and hydroxyl group position due to different resonance stabilization (17). In addition to antioxidant activity, phenolic acids offer antimicrobial activity, which is determined by the number, position of substitution in the benzene ring, and chemical structure (activity increased as the length of the alkyl chain increased) (18).

1.3 Amino acids as antioxidants

Reactive oxygen species (ROS) and free radicals may come from internal sources, such as normal metabolic processes, phagocytic cells, mitochondria, inflammation, peroxisomes, and exercise. Or it may come from external sources, including environmental pollutants, cigarette smoke, ultraviolet light, radiation, pesticides, alcohol consumption, Ozone, and viral infections. Figure 1.2 shows the different sources of free radicals formation (19).

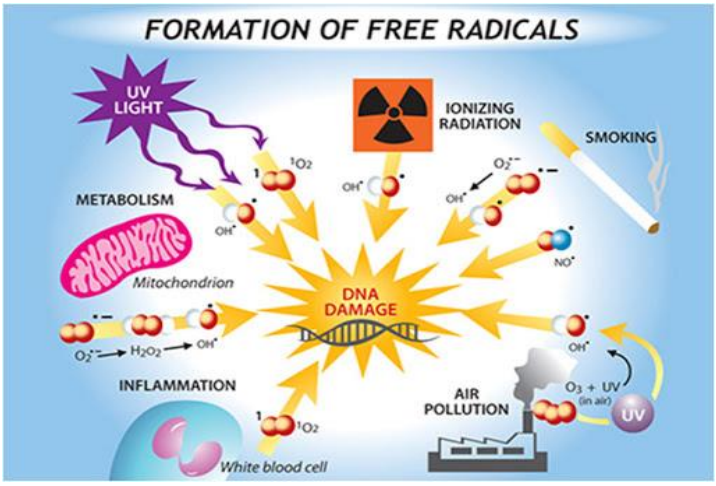


Figure (1.2): Different sources of free radicals (20)

The imbalance between the free radical's production and antioxidant protection systems leads to oxidative stress, which has a role in apoptosis,

aging, and other cellular processes. In the balanced cell, the level of reactive oxygen species generated as a by-product of normal metabolic processes can be controlled by antioxidants like vitamin C and vitamin E. On the other hand, imbalance leads to an increase in enzyme deactivation oxidative DNA damage, and lipid peroxidation damage, which lead to much oxidative related disease, such as diabetes, heart disease, cancer, and macular degeneration disease. For this reason, there is a growing interest in the action of antioxidant compounds, and whether they can bind free radicals in human body, therefore reducing cellular damage (21, 22).

Antioxidant compounds are defined as substances that inhibit oxidation or protect materials from auto-oxidation. Thus, these substances can reform damage carried out by ROS or free radicals, and therefore, reduce aging and risk of cancer. Mechanism of action of antioxidant compounds include scavenging free radicals, absorbing UV radiation, binding metal ion catalysts, and produce a non-radical species from hydroperoxides or intercepting single oxygen (23). Amino acids are often used as antioxidants; there are many methods that amines provide for antioxidant activity, for example, radical scavenging ability such as butylated hydroxytoluene (BHT) and tocopherols or chelation of metals (24). Arginine, histidine, and lysine are considered amino acid examples that possess a potent antioxidant activity (25).

1.4 Amino acids as antimicrobial agents

Microorganisms or microbes are unicellular or multicellular microscopic organisms such as bacteria, fungi, protozoa, viruses, slime molds, and algae. Microbes can be found in the air, soil, water, and humans body (26).

Millions of microorganisms present in the body, some of them are essential for human health. However, others caused illness. Microbial infection is the infestation of body tissues by disease microbes, multiplication, and body tissue's reaction with the infectious agents. Then, it can be destructive and sometimes mortal to the host. The scarcity of antimicrobial drugs and the rise of antibiotic resistant microrganisms have tormented human lives for a considerable length of time. Most of the present drugs failure is the result of microbsability to recognize the mechanism of action of exiting drugs and developing resistance to them, through various mechanisms. For this reason, recently, there has been increasing attention in researching and improving novel antimicrobial agents from diverse sources to face microbial resistance (27, 28).

Antimicrobial drugs play an essential role in killing or inhibiting the growth of microbes, particularly pathogenic microorganisms (29). These medications can be classified according to the type of microorganisms they act on. For instance, drugs that are used against bacteria are called antibacterial. Ones used against the fungi, are called antifungal. They are also classified as microbicidal if the microbe is killed and biostatic if the drug inhibits the microorganism’s growth (30).

Infectious illness caused by microbes, mainly fungi and bacteria considered a major international health problem. Bacteria and fungi are becoming very drug-resistant that led to issues with drugs. Several types of resistant bacterial and fungal infections are difficult to treat, increasing morbidity and death rate (31). Amino acids, amino acids derivatives, ester, and amide form various substances with various biological and pharmacological properties. These compounds prove their activity against *Candida albicans; Candidakrusei*; *Candida glabrata; Candida guilliermondii*; *Pseudomonas aeruginosa*; *Staphylococcus aureus, Escherichia coli*, *Proteus vulgaris, Klebsiella pneumonia (32).*

1.5Chromatography

Chromatography and separation techniques are essential for analysis and compounds synthesis. They enable the identification, separation, and purification of the mixture components for quantitative and qualitative analysis (33). Separation occurs according to component size, charge, binding affinity, polarity, and other properties (34).

High-performance liquid chromatography (HPLC)

It is the most common analytical technique for qualitative and quantitative analysis of liquid samples. It consists of a mobile phase which is a solution pumped through the stationary phase (column). The separation principle of HPLC depends on the different solubility of sample components between two phases (35).

Thin liquid chromatography (TLC)

A type of chromatography, that is used to separate the non-volatile mixture components through the thin stationary phase. The separation process occurs according to the competition between the stationary and solvent systems (mobile phase) to adsorption of solute (36).

Gas chromatography (GC)

Martin designed the gas chromatography technique. In this method, the process of components separation from mixture depends on the partition between the stationary phase (liquid) and mobile (phase gas) (37).

1.6 Spectroscopy

Infrared spectroscopy (IR)

Infrared spectroscopy helps analyze samples present in any form, either solid, gas, or liquid. In IR spectroscopy, the existing substance in the sample, interacts with the electromagnetic spectrum in the infrared (IR) region. Electromagnetic radiation (EMR) absorption in the infrared region depends on resonant frequency and group's matches with that frequency (38).

Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance spectroscopy is one of the most important analytical techniques used in research and quality control for defining the sample content, purity, and molecular structure (39).

Carbon-13 (C^{13}) nuclear magnetic resonance

This type of spectroscopy is most commonly known as ^{13}C NMR or carbon-13 NMR, one of the analytical tools in organic and biological sciences. It has the ability to identification carbon atoms in organic molecules (40).

1.7 Literature review

In previous researches conducted by the researcher groups, explain that the structure is not essential for the activity (41). However , other researchers mention that, the catechol moiety with a hydroxyl group in positions 3 and 4 is vital for antioxidant activity through free radicals scavenger for this form of phenolic compounds (42, 43). There is a study proving that the 4-hydroxyl benzoic acid and 4- hydroxyl cinnamic acid have antimicrobial activity (44). In addition to that, there is a study contains a reaction of mono hydroxybenzoic acids and benzoic acid with 2-phenoxyethanol to produce mono acids ester compounds, which offer anticancer and antifungal activities. In 2019 there is a study on the synthesis of thio-acid esters series, which showed high biological activity in most tests, especially in lipase, anticancer, and antimicrobial activities.

Moreover, there is a series of amino acid esters, whose biological activity neither studied.Therefore, it is important to synthesize these esters, examine its biological activities, and compare them with different positions of hydroxyl group on the acid esters moieties.

1.8 Aim of the thesis

The main objectives of this thesis are the following:

- ➢ To synthesize a series of the substituted phenolic acid ester by reaction with 2-phenylaminethanol.
- ➢ To explore the biological activities of these amino-acid esters.
- ➢ To enrich the literature with the physical data of these series of amino-acid esters.

Chapter Two

Materials and methods

2.1 materials and chemicals

The following materials and chemicals were used: α-tocopherol, (DPPH), benzoic acid, salicylic acid, 3- Hydroxybenzoic acid, 4- Hydroxybenzoic acid, 2- chloroethanol, aniline,β-carotene, and linoleic acid were purchased from Sigma, (Sigma, Aldrich GmbH, Sternheim, Germany). While chloroform, ethanol, Tween-40, sodium carbonate, Folin-ciocalteu's phenol reagent (FCR), ethyl acetate, and other chemicals reagents were purchased from Merck (Darmstat, Germany). RPMI 1640 culture medium, Trypsin, glutamine, fatal calf serum, amphotricine B, Trypan blue solution, Hank's balanced solution, gentamicin, and penicillin other reagents will be of analytical grade.

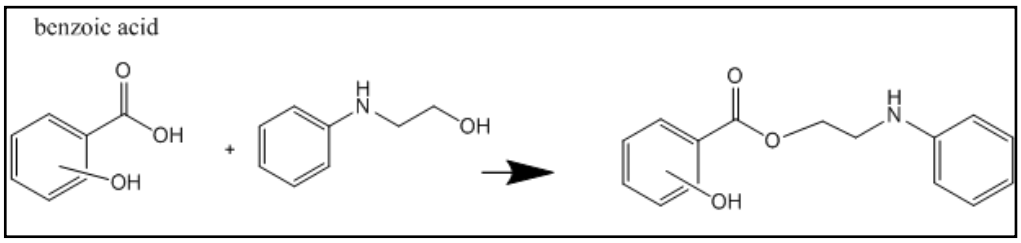
Concerning the microorganisms under the microscope, included bacteria were *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 9027), *Proteus vulgaris* (ATCC 8427), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538), MRSA (Clinical sample). On the other hand, the fungi included in this study were *candida albicans* (ATCC 90028). All previously tested microorganisms were obtained from Biodiversity & Environmental Research Center (BERC) Til Village-Nablus.

2.2 Physical measurements

Each compound's melting range was determined using the start melting point apparatus, R00102618, while IR for each compound was detected by infrared spectrophotometer (Necolet Is5 - Id3) at An-Najah University. ^{1}H –NMR and Carbon13 were determined by (Bruker 500 MHz-Avance III) at the University of Jordan/ Jordan. The purity of the compounds was confirmed by HPLC Breeze 2 HPLC system.

2.3 General procedure for the synthesis of amino-acid esters

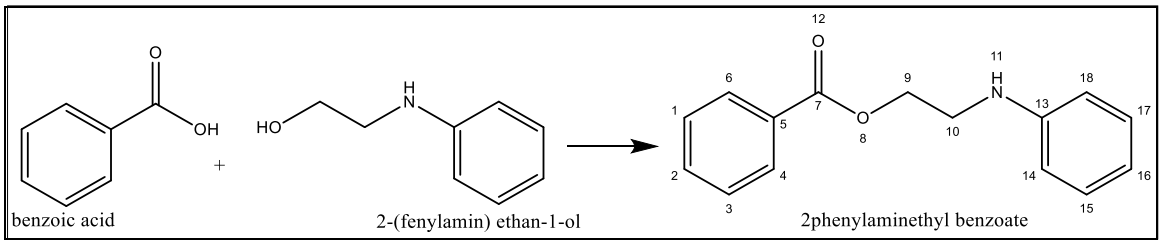
Amino-acid esters were produced by refluxing benzoic acid and its derivatives with 2-phenylaminethanol for 120 minutes. The mixture was left at room temperature for 24 hrs. Each compound's reaction product was dried from the solvent. The residue was purified by flash chromatography on a silica gel using (n-hexane /ethyl acetate) in the ratio 8:2, respectively. The following scheme 2.1 shows the general reaction for amino acid ester synthesis, where OH is substituted in Ortho, Meta, and Para positions.



Scheme 2.1: General reaction for amino-acid esters synthesis.

2.3.1 Preparation of 2-phenylaminethyl benzoic acid (I)

The 2-phenylaminethyl benzoic acid was prepared by mixing 10 ml of 2-phenylamine ethan-1-ol with benzoic acid (3 g, 0.025mol) for two minutes. Two drops of concentrated HCL were added and refluxed for 2 hours, then left for a night at room temperature. The solvent was dried from the product and then purified by flash chromatography on a silica gel by using (n-hexane /ethyl acetate) in a ratio of 8:2, respectively. Scheme 2.2 shows reaction for preparation 2-phenylaminethyl benzoic acid.



Scheme 2.2: Reaction of benzoic acid with 2-phenyl amine ethan-1-ol.

Percentage yield is (72%), MP equal (194-198) and λ_{max} = 240- 275 nm

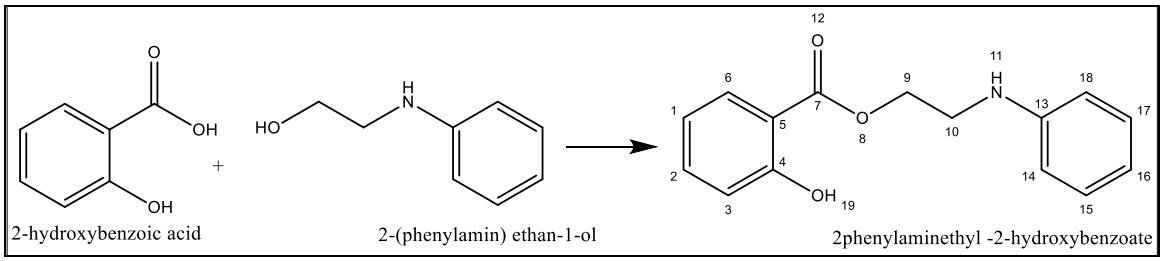
IR ν_{max} (O-C 2847.9, C=O 1685.5,N-H 1454, ester 1423.5 1242, CH_2- 667.5 cm^{-1}).

^{1}HNMR ($CDCl_3$)δ: H1,H2,H3,H4,H6 (7.55-8.06) ; H13, H14,H15,H16, H17 (6.58-7.07); 2H9, 2H10 (2.3-3.3); N-H (9.53) ppm

^{13}CNMR (CDCl3) δ: C7 (156);C9,C10 (40); C1, C2, C3, C4, C5, C6(128-133); C12, C13, C14, C15, C16, C17 (113-140) ppm.

2.3.2 Preparation of 2-phenylaminethyl - 2- Hydroxybenzoic acid (II)

The 2-phenylaminethyl- 2- hydroxy benzoic acid was prepared by mixing 10 ml of 2- phenylamineethan-1-ol with 2- hydroxy benzoic acid (3.1 g, 0.022 mol) for two minutes. Two drops of concentrated HCL were added and refluxed for two hours, then left for a period of the night at room temperature.The solvent was dried from the product, and then purified by flash chromatography on a silica gel by using (n-hexane /ethyl acetate) in ration 8/2 respectively. Scheme 2.3 shows reaction for preparation 2-phenylaminethyl- 2- hydroxybenzoic acid.



Scheme 2.3: Reaction of 2-hydroxy benzoic acid with 2-phenyl amine ethan-1-ol.

Percentage yield is (68%), MP equal (185.0-187.7) and λ_{max} = 235-270 nm

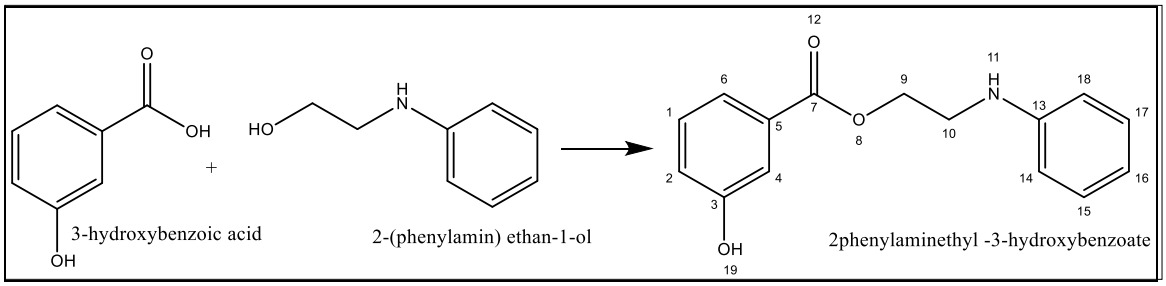
IR: O-H, O-C (2822.3); C=O (1674.7); N-H (1461.8); -COO (1422.8; $-CH_2$ (686.6) cm^{-1}.

^{1}HNMR ($CDCl_3$) δ: H1, H2, H3, H6 (6.87- 7.82); H15, H16, H17, H18, H19 (6.58-7.07); 2H4, 2H10 (2.1-3.8); N-H (9.5); O-H (10.5) ppm.

^{13}C-NMR ($CDCl_3$ δ: C7 (165); C9, C10 (40); C1, C2, C3, C4, C5, C6 (116-150); C12, C14, C15, C16, C17, C18 (113- 140) ppm.

2.3.3 Preparation of 2-phenylaminethyl - 3- Hydroxybenzoic acid (III)

The 2-phenylaminethyl- 3- Hydroxybenzoic acid was prepared by mixing 10 ml of 2- phenylamineethan-1-ol with 3- Hydroxybenzoic acid (3.09 g, 0.022 mol) for two minutes. Two drops of concentrated HCL were added and refluxed for 120 minutes, then left for a night at room temperature. The solvent was dried from the product and then purified by flash chromatography on a silica gel by using (n-hexane /ethyl acetate) in the ratio of 8/2, respectively. Scheme 2.4 shows reaction for preparation 2-phenylaminethyl- 3- Hydroxybenzoic acid.



Scheme 2.4: Reaction of 3-hydroxy benzoic acid with 2-phenyl amine ethan-1-ol.

Percentage yield is (88%), MP equal (195-198) and λ_{max} = 240 - 265 nm

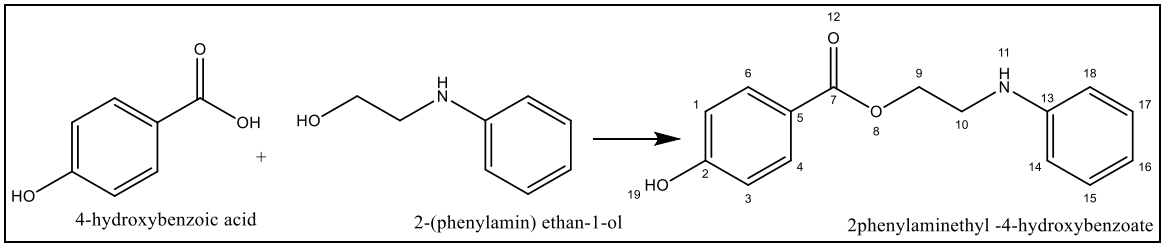
IR ν_{max} : O-H (3394); O-C (2884); C=O (1683); N-H (1455); -COO (1419); $-CH_2$ (634) cm^{-1}.

^{1}HNMR ($CDCl_3$):δH1, H2, H4, H6 (7.13-7.59; H14, H15, H16, H17, H18 (6.58-7.07); 2H9, 2H10 (2.2-3.8); N-H, O-H (10.5) ppm.

^{13}CNMR ($CDCl_3$): C7 (165); C9, C10 (140); C1, C2, C3, C4, C5, C6 (117-135); C13, C14, C15, C16, C17, C18 (114-140) ppm.

2.3.4 Preparation of 2-phenylaminethyl - 4- Hydroxybenzoic acid (IV)

The 2-phenylaminethyl- 4- Hydroxybenzoic acid was prepared by mixing 10 ml of 2- phenyl amine ethan-1-ol with 4- Hydroxybenzoic acid (3.002 g, 0.022 mol) for two minutes. Two drops of concentrated HCL were added and refluxed for 120 minutes, then left for a night at room temperature. The solvent was dried from the product and then purified by flash chromatography on a silica gel using (n-hexane /ethyl acetate) in the ratio of 8/2, respectively. Scheme 2.5 shows reaction for preparation 2-phenylaminethyl- 4- Hydroxybenzoic acid.



Scheme 2.5: Reaction of 4-hydroxy benzoic acid with 2-phenyl amine ethan-1-ol.

Percentage yield is (85%), MP equal (193.0-196.0) and λ_{max} = 240-270 nm

IR ν_{max} : O-H (3385); O-C (2883); C=O (1688); N-H (1454); -COO (1425); -CH_2(667) cm^{-1}.

^{1}HNMR ($CDCl_3$) δ: H1, H3, H4, H6 (6.8-7.8); H14, H15, H16, H17, H18 (6.6-7); 2H2, 2H10 (3.8-4.7); N-H (8.5); O-H (9.7) ppm.

^{13}C-NMR ($CDCl_3$) δ: C7 (165); C9, C10 (40); C1, C2, C3, C4, C5, C6 (116-150); C12, C14, C15, C16, C17, C18 (113-140) ppm.

2.4 Checking for purification

The purity of the samples was confirmed by HPLC (Breeze 2, HPLC system. The mobile phase was methanol and acetonitrile (60:40). Figures showed the retention time and area under the peak. The retention time of Benzoic acid was 2.6 and purity 99.5%, 2-hydroxy was 2.54 and 98.6%, 3-hydroxy 2.49 and 99.97%, and 4-hydroxy 2.49 and 99.99 % purity.

2.5 Antioxidant properties

As mentioned before, antioxidants are stable compounds or molecules that can give electrons to the free radicals, thus becoming less dangerous, less active, and decreasing damage. These antioxidant compounds have a free radicals scavenger property, contributing to inhibiting or delaying cellular damage (45). Antioxidant compounds can remove the unpaired radical by equalizing free radicals by donating or accepting electrons and antioxidants known as substances that can inhibit the oxygen-mediated oxidation of various substances (46). In this research, antioxidants activity was examined through two methods, the first was a DPPH assay, and the second was β-carotene- linoleic acid.

2.5.1 DPPH assay

Four aromatic synthetic compounds were tested for the efficiency of scavenging free radicals matched with Trolox as a basic. 0.004% methanol solution of DPPH and different concentrations of the compounds and Trolox in methanol were prepared. Then one ml of compound solutions was added

to 4 ml of 0.004% methanol solution of DPPH. After 30 minutes incubation period at room temperature, the absorbance was read against a blank at 517 nm.And the inhibition percentage I (%) of free radicals through DPPH of each compound and Trolox was calculated by the following equation

$$I\ (\%) = ((A\ blank - A\ sample)/ A\ blank) \times 100 \qquad \text{Equation (1)}$$

Where;

A blank:Absorbance value of the control reaction containing all reagents except the synthesized compound.

A sample: Is the absorbance value of the synthesized compound.

Test compound concentrations that giving 50% inhibitions (IC_{50}) were calculated via the plot of inhibition (%) against test compounds concentration. Trials were carried out in triplicates.

2.5.2 β- carotene- linoleic acid assay

β- carotene- linoleic acid bleaching assay is a well-known method for estimating the antioxidant activity developed by Miller and Gizzani (4). This spectrophotometric technique monitors carotenoid bleaching obtained by its interaction with peroxyl radicals generated through the oxidation of linoleic acid (47). The nature of the reaction that occurs leads to a resonance firm radical by adding the peroxyl radical to the carotenoid polyenic system. This center radical carbon can produce nonradical products by reacting with other radicals; furthermore, it can create a highly reactive carotene peroxyl radical

by adding molecular oxygen in a quick process. The consumption of the carotenoid is reduced by the addition of a radical scavenger, as a function of the antioxidant concentration (48, 49).

The working principle of the β- carotene linoleic acid bleaching assay is based on the β-carotene solution's discoloration because of the broken π-conjugation. The free radical species is produced from linoleic acid autoxidation by incubated at 50 °C. When a suitable antioxidant is added to the β-carotene solution, the discoloration can be delayed through competition for a reaction between antioxidant and β-carotene with radicals (50).Briefly, 1 mg of β-carotene was dissolved in 20 mg of linoleic acid, 2 ml chloroform, and 200 mg of Tween 80 were added. Chloroform was evaporated by using a rotary evaporator under low pressure at a temperature less than 30 °C. And 200 ml of oxygenated distilled water was added to the flask with strong shaking for 30 minutes. Then 5 ml of the prepared emulsions were transferred to tubes, each containing 0.1 ml of compound solution or tocopherol with 2 mg/ml consecration.

A control sample was prepared precisely as before but without adding antioxidants.Each type of sample was prepared in triplicate. The test systems were placed in a water bath for 2 hours at 50 °C.The value of absorbance of each sample was read by ultraviolet spectrometry at $\lambda = 470$ nm, immediately after sample preparation and at 15-min intervals until the end of the experiment ($t = 120$ min). Antioxidant activities in the β-carotene linoleic acid model were measured by the changes in the absorbance at 470 nm.

2.6 Anti-lipase enzyme

Lipase is an enzyme, secreted by pancreas to break down dietary fat. It structurally, belongs to the secondary class of esterases. Activity of lipases found in the intestine can be decreased by a substance called Lipase inhibitor. The lipase inhibitors, reduce lipid digestion through inhibition of Lipase, with the resultant reduction of the absorption of fat from gastrointestinal tract, and its excretion with feces. Reduced fat absorption will lead to weight loss. Therefore, lipase inhibitors are used to treat obesity, which is a major risk factor forcardiovascular diseases and Type II diabetes (51, 52).Stock solution (DMSO 10%) and different concentrations of each synthesized compounds and Orlistat (control) were prepared (200, 400, 600, 800, 1000) µg/ml. Then 0.1 ml of lipase with 1 mg/ml concentration was added to a series of a tube that contains 0.2 ml of test compound solution.

Tubes were completed to 1 ml of tri- HCl and incubated for 15 min at 37 °C. After that, 0.1 ml of PNPB (p-nitrophenyl butyrate) added to each test tube and incubated again for 30 min at 37 °C. The value of absorbance of each sample was read by ultraviolet spectrometry at λ= 410 nm.The Inhibition percentage I (%) of test compounds and Orlistat was calculated by the following equation

$$I\ (\%) = ((A\ control - A\ sample\ test)/ A\ control) \times 100$$

Equation (2)

2.7 α-Glycosidase activity

Alpha-glycosidases are enzymes that hydrolyze glycosidic bonds of disaccharides to produce simple sugars. In the human body, these enzymes contribute to carbohydrate digestion to generate glucose, available for intestinal absorption, which leads to an increase in blood glucose level. AGIs (Alpha-glycosidase inhibitors) or starch blockers are compounds that block the carbohydrates absorption from the gut and can be used for the treatment of type 2 diabetes or glucose intolerance. Currently, there is no evidence, that AGIs prevent macro- or micro vascular complications, or improve survival in type II diabetes (53, 54).

To perform α- glycosidase activity**,** 50 µl of phosphate buffer (100 mM) at PH = 6.8 was prepared. And 20 µl of varying concentration of test compounds and α-glycosidase were prepared (0.1, 0.2, 0.3, 0.4, 0.5) mg/ml.Then incubated for 15 min at 37 °C. 20 µl of PNPG was added to the substrate and then incubated for 20 min at 37°C. For stop reaction 50 µl of Na_2CO_3 was added. The value of absorbance of each sample was read by ultraviolet spectrometry at λ= 405 nm. The Inhibition percentage I (%) of test compounds was calculated by the following equation

$$I\ (\%) = ((A\ control - A\ sample\ test)/ A\ control) \times 100$$

Equation (3)

2.8 Antimicrobial activity

Broth micro dilution method was used to determine the antibacterial and antifungal activity of the synthesized compound against the following strain: *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 9027), *Proteus vulgaris* (ATCC 8427), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538), MRSA (Clinical sample) and *Candida albicans* (ATCC 90028) by the following procedure:

A swab was taken from the different types of bacteria and fungus then placed in normal saline. Turbidity was measured using the UV at $\lambda = 620$, it should be between 0.08 - 0.12. If it is less than that, bacteria are added, and if it is greater than this value, normal saline is added. After that, 100 µl from each type of bacteria added to tubes contained 10 ml of Mueller–Hinton broth attended previously. A solution of each test compound in DMSO 10% (40 mg/ml) was prepared. For all bacteria were tested, we had three controls: 1)positive control which contains media and bacteria, 2) negative control which only contains media, 3) compounds control (compound+ media) to be sure that there is no contamination or turbidity, and that the change is not due to the compound itself. So compounds were serially diluted in this control. Antibacterial activity measured by 96-well disposable plastic trays after incubated for 24 h. All tests were performed in triplicates.

2.9Anticancer activity

Cytotoxicity Method

RPMI-1640 media is used to culture the cervical adenocarcinoma (HeLa) cancer cells. We added for this media 10 % of fetal bovine serum, 1 % penicillin/streptomycin, and 1 % L-glutamine.The Hela cells were grown in the humidified environment at 37 °C with 5 % CO_2 atmosphere, then we used a 96-well plate to seed the cells at 5×10^3 cell/well. After 24 hours, cells were treated with various concentrations of the tested compounds for 48 h. Cell viability was assessed by the Cell-Tilter 96® Aqueous One Solution Cell Proliferation (MTS) assay according to the manufacturer's instructions (Promega Corporation, Madison, WI). Briefly, at the end of the treatment, 20 μL of MTS solution per 100 μL of media was added to each well and incubated at 37 °C for 2 h. Absorbance was measured at 490 nm. The compounds were tested in vitro against Hela cell type using the same test and procedure.

Chapter Three

Results and Discussion

3.1 DPPH assay resultDPPH **(** α, α-diphenyl-β-picrylhydrazyl), (DPPH; $C_{18}H_{12}N_5O_6$, *M* = 394.33) is a simple, economical, rapid, and widely applied procedure to estimate antioxidant activity by measure the ability of compounds to work as hydrogen donors or free radical scavengers. The DPPH free radical is organic nitrogen radical. It is commercially available with dark purple color and does not need to be produced before the assay. The molecule of DPPH is described as a stable free radical under the spare electron's delocalization over the molecule as a whole. Thus, the molecules do not dimerize. The delocalization leads to appearing the deep violet color described as having the potential to absorption in methanol solution concentrated at about 517 nm. When DPPH solution is mixed with that substance, it donates a hydrogen atom and gives rise to the reduced form and loss of the dark violet color (55, 56). The results for the DPPH assay of compounds and Trolox are shown in the table 3.1 and figure 3.1.

Table 3.1: Percent inhibition of radicals by benzoate compounds.

Concentration (mg/ml)	**% inhibition**					
Compound	I	II	III	IV	Trolox	
0	0	0	0	0	0	
0.25	66.2	63.5	64	65.4	95.8	
0.5	67.4	65.7	65	68.1	95.8	
1	67.3	66.4	65.5	74	94.4	
2	68.2	69.4	66.1	79.3	93.7	
IC_{50}	0.0017	0.0013	0.0011	0.0009	0.0001	

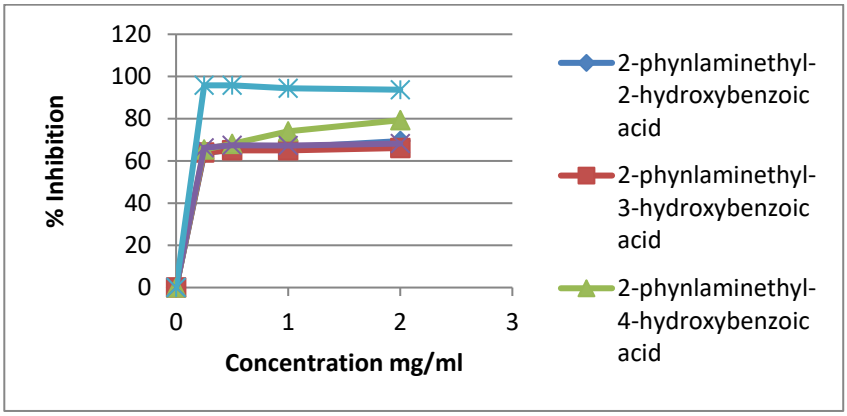


Figure 3.1: percent inhibition of radicals by benzoate compounds

The DPPH results show that, the four synthesized compounds have an excellent free radical scavenging activity, especially compound IV derived from 4-hydroxy benzoic acid at a concentration of 2 mg/ml. This is a high activity near to Trolox action at the same concentration.

At the same time, there is a little difference between the values of percentage inhibition of different compounds, which means that, there is slight effect on the antioxidant activity with change of position of functional group as ortho, Para and Meta. This is in line with the results of previous studies that have

proven that the structure of phenolic compounds did not affect the antioxidant activity. The IC_{50} for all synthesized compounds > 0.001 mg/ml, while for Trolox is about 0.0001 mg/ml. Our results are in good agreement with literature done on polyphenlic compounds like aromatic thio-acid esters, which showed that the compounds have good free radical scavenging activity and IC_{50} for thio-acid esters > 0.4 mg/ml (61).

3.2 β- carotene- linoleic acid assay

The same synthetic aromatic compounds were tested for their antioxidant activity using the emulsion system of β- carotene linoleic acid relied on the fact that in the absence of antioxidant, the β- carotene loses its color (49).The results for the β- carotene- linoleic acid assay of compounds are shown in table 3.2 and figure 3.2.

Table 3.2: Percent inhibition of tested compounds for β- carotene test

Time (min)	**Absorbance**				
compound	I	II	III	IV	Tocopherol
0	0.835	0.845	0.807	0.65	1.2
15	0.641	0.482	0.63	0.421	1.1
30	0.528	0.31	0.492	0.275	1.09
45	0.488	0.221	0.308	0.1846	1.08
60	0.439	0.144	0.22	0.101	1
75	0.403	0.133	0.196	0.064	0.8
90	0.371	0.116	0.166	0.061	0.9
105	0.349	0.11	0.123	0.0613	0.9
120	0.342	0.1006	0.0906	0.06	0.85

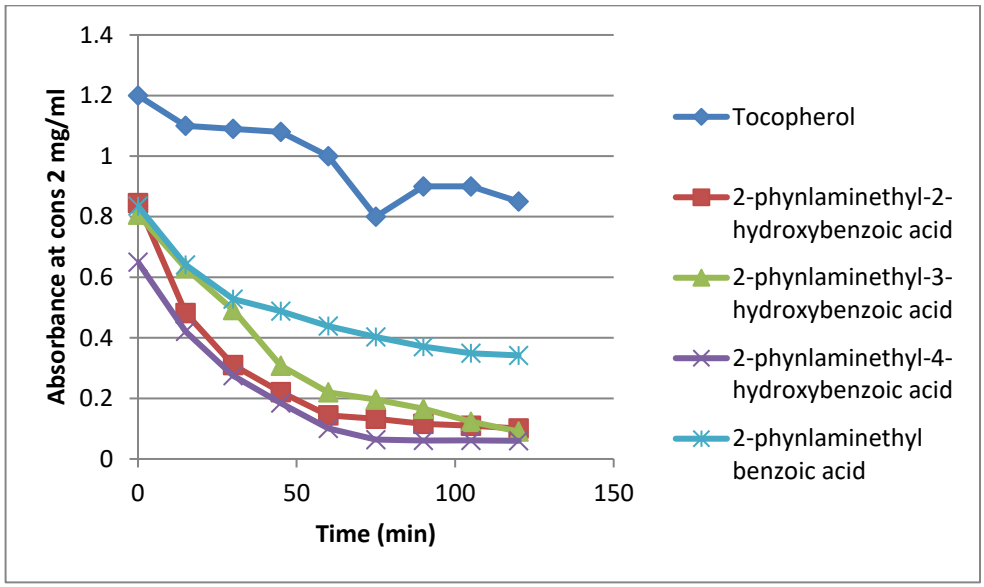


Figure 3.2: Percent inhibition of tested compounds for β-carotene test

The fact that β-carotene loses its color in the absence of antioxidants, the same compounds were tested for their antioxidant activity using the emulsion system of β-carotene linoleic acid.

At concentration 2 mg/ml, results show that, the synthesized compound from benzoic acid and 3- hydroxyl benzoic acid,has antioxidant activity value closer to the alpha-tocopherol, compared with compound synthesis from 2 and 4 hydroxy benzoic acid. When comparing this result with the results of the related study, we find that the synthesized compound derived from benzoic acid showed higher antioxidant efficiency compared with the other synthetic compounds from benzoic acid derivatives. The antioxidant activity decreases gradually with time increasing for all compounds, which also in line with the results of previous studies. Throughout the time, compound I possess the best antioxidant activity. On the other hand, compound IV was found to be less effective. Compound II which was derived from 2-hudroxy benzoic acid, showed higher activity compared with the compound III after

105 minutes. When comparing these results with the results of the thio-acid esters compounds, we find that these compounds showed 2-thiophenylethanol benzoate revealed the best antioxidant with absorbance 1.04 after 75 min compared with the other synthetic compounds (61).

3.3 Anti-lipase enzyme

The effect of the pancreatic lipase was reported by measuring the hydrolysis of p-nitrophenolate to p-nitrophenol at 410 nm using the UV spectrophotometer device. The results of percentage inhibition and IC_{50} values for the aromatic synthetic compounds and Orlistat are shown in table 3.3 and figure 3.3.

Table 3.3: Percent inhibition of tested compounds and Orlistat for anti-lipase.

Concentration (µg/ml)	**% Inhibition**				
compound	I	II	III	IV	Orlistat
0	0	0	0	0	0
200	50.3	44.7	39.9	40.1	92
400	48.5	44.3	41	39.1	93.5
600	47.9	43.7	41.7	37.8	95.1
800	48.9	42.5	41.6	42.8	97.2
1000	48.3	42.7	38.5	42.4	98.6
IC_{50}	202	215	228	231	98

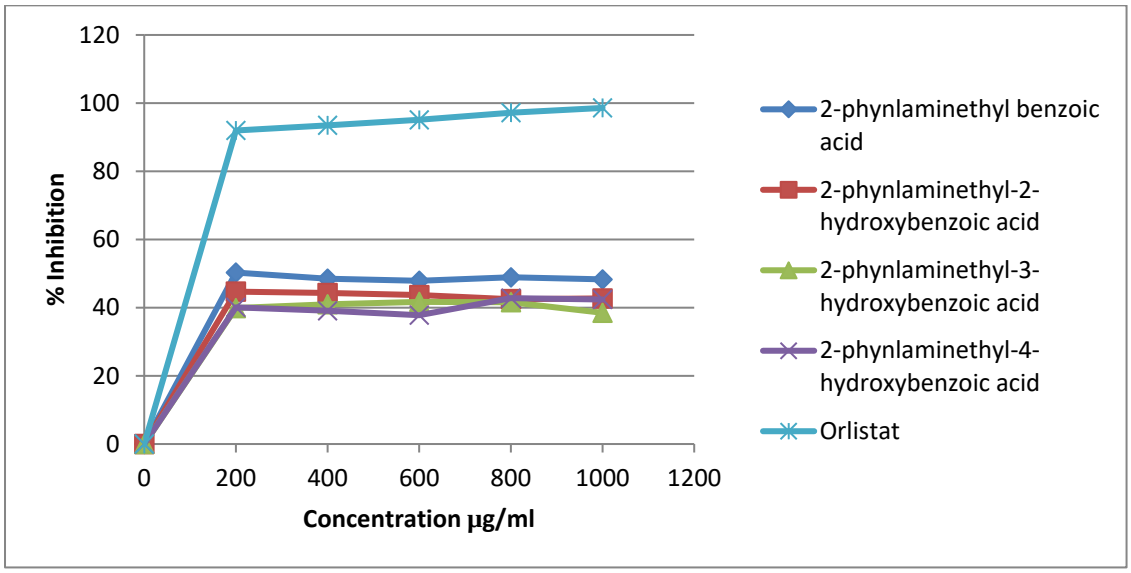


Figure 3.3: Percent inhibition of tested compounds and Orlistat for anti-lipase.

The test measures the percent inhibition of the compounds compared to the drug orlistat , used as a standard. Table 3.3 and Figure 3.3 showed the percent inhibition and IC_{50} result for the compounds and orlistat. Compound one show the best results of percentage inhibition, and it has IC_{50} value at concentration 202 µg/ml. Compound IV which has IC_{50} 231µg/ml, showed the least anti lipase activity. Generally, all synthesized compounds did not demonstrate a high anti lipase activity; this is possibly due to the lack of amide group, which is present in orlistat. The same result was founded in all polyphenolic compounds doesn't contain amide group (61).

3.4 α-Glycosidase activity

Table 3.4and figure 3.4 show the percent inhibition for the α-glycosidase enzyme and IC_{50} values of the synthesized aromatic compounds compared with standard compound (Acarbose).

Table 3.4: Percent inhibition of tested compound for α-glycosidase

Concentration (µg/ml)	**% Inhibition**				
compound	I	II	III	IV	Acarbose
0	0	0	0	0	0
100	74	77.4	76.7	78	68.6
200	75	78.2	80.5	79.8	72.3
300	77	79.6	80.9	80.3	85
400	82	82.2	84.2	81.1	93.8
IC_{50}	82.14	84.5	87.26	81.7	83

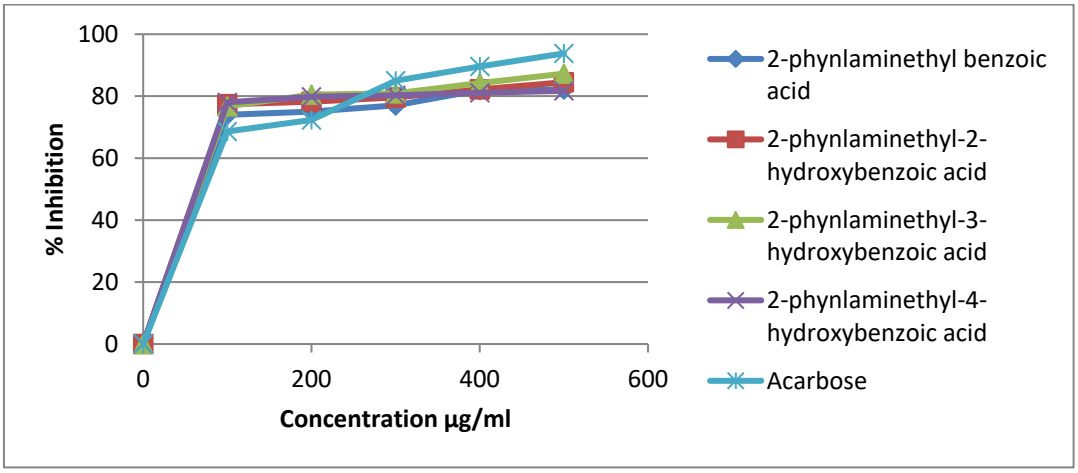


Figure 3.4: Percent inhibition of tested compound for α-glycosidase.

Table 3.4 shows the percent inhibition and values of IC_{50} of the synthesized acid esters compared with Acarbose (standard compound). Compounds I and IV showed IC_{50} values 82.14 and 81.7 µg/ml respectively, which is more effective against enzyme Acarbose IC_{50} is 83, while compounds II and III showed IC_{50} 84.5, 87.26 µg/ml respectively. It is noted that the percent

inhibition was increased as the concentration of the compound increased. The results are justified and reasonable due to the synthesized aromatic compounds are modified acid esters and have the same functional groups as phenolic compounds, such as Trans- p-coumaric acid.

3.5 Antimicrobial activity

The four aromatic compounds were tested against six bacteria and one fungus that cause dermic and mucosal infections in humans (57). The minimum inhibitory concentration (MIC) values of synthetic compounds and references' (Fluconazole, Ampicillin, and Ciprofloxacin) are shown in table 3.5.

Table 3.5: Microbial growth MIC values of synthesis compounds (mg/ml)

Microbe	*K.pneumoniae*	*P. aeruginosa*	*P. vulgaris*	*S. aureus*	*E. coli*	MRSA	*C.*albians
ATCC	13883	9027	8427	25923	25922	Clinical sample	90028
I	2.5	2.5	2.5	1.25	5	2.5	2.5
II	1.25	1.25	0.625	1.25	2.5	1.25	2.5
III	4	2	2	1.25	4	1.25	4
IV	2	2	2	1.25	2	1.25	2
Fluconazole	R	R	R	R	R	R	1.62
Ampicillin	0.002	2.01	0.017	2.12	2.12	R	R
Ciprofloxacin	0.132	0.016	2.25	0.87	1.45	0.15	R

The four synthesized compounds showed antibacterial activity with varying degrees. Compound II and compound IV showed significant activity at a concentration of 0.625-2.5 mg/ml and 1.25-2 mg/ml, respectively, against all tested bacteria and fungi. The compound I showed good activity against all bacteria at concentrations 1.25-2.5 mg/ml. However, it was not effective towards *E.coli* as concentration was required to appear the inhibition was 5 mg/ml. The only compound that did not show activity against *Candida* is compound III, which required a concentration of about 4mg/ml to inhibit inhibition. Also, the same concentration is required against *E.coli* and *Klebsiella*.

When we compared the MIC for all tested compounds with effective antibiotics, we found that compound I has a MIC better than MIC of Ampicillin against *S. aureus* bacteria, as shown in table 4.5. *Candidas* resistant toward Ampicillin and ciprofloxacin, but 2.5mg/ml from compound I enough for good inhibition. Compound II and III have a MIC better than MIC of Ampicillin against *S. aureus* and *pseudomonas aeruginosa* bacteria. Ampicillin has a MIC of about 2mg/ml, but MIC for compounds II and III about 1.25 mg/ml.As a comparison with other polyphenolic compounds, the results of aromatic esters compounds from 2-phenoxyethanol were negative and there was no activity against any of the tested types of bacteria (61).

Despite the apparent reduced antibacterial effect of these compounds compared to known antibiotics, these results are encouraging, as the need for

new antibiotics is increasing globally, because of emergence of antibiotic-resistant micro-organisms.

3.6 Anticancer activity

Tables 3.6, 3.7, 3.8, and 3.9 show the activity of benzoate compounds against Hela cancer cells were measured by cytotoxicity method (MTS assay). MTS assay is a rapid, sensitive, economic, and specific in vitro cytotoxicity assay (58).

Table 3.6: Compound I activity against Hela cancer cells.

Concentration (µg/ml)	500	250	125	62.5	31.25
control					
0.962	1.026	0.999	0.987	1.008	0.928
0.914	1.001	1.003	1.059	0.927	0.937

Table 3.7: Compound II activity against HeLa cancer cells.

Concentration (µg/ml)	500	250	125	62.5	31.25
control					
0.962	1.033	0.975	0.779	0.721	0.897
0.914	1.12	0.972	0.854	0.897	0.971

Table 3.8: Compound III activity against HeLa cancer cells.

Concentration (µg/ml)	500	250	125	62.5	31.25
control					
0.962	0.99	1.067	1.191	1.239	1.251
0.914	1.061	0.987	1.222	1.242	1.345

Table 3.9: Compound IV activity against HeLa cancer cells.

Concentration (µg/ml)	500	250	125	62.5	31.25
Control					
0.962	0.991	1.195	1.226	1.486	1.531
0.914	0.992	1.168	1.368	1.334	1.469

The result didn't show significant activity toward cancer cells (Hella type). The best compound was II which showed 25% activity. Another important note that compound IV activates the growth of the cells at concentration 31.25 µg/ml. Those results are not amazing. Activities of compounds may be attributed to the molecular structure and the position of functional groups. Several previous studies have showed that catechol moiety with 3, 4-hydroxy is important for free radical activity. On the other hand, other studies suggested that the structure of the compounds is not important for the activity (59, 60).

Chapter Four

Conclusion

The synthetic compounds were prepared efficiently by the reaction of acid esters and 2-phenylaminethanol. The compound's identity was confirmed using a spectroscopic method such as ^{1}HNmr, UV, carbon 13, and IR. The amino acid ester compounds were identified depending on some physical properties such as thin-layer chromatography and melting points. Many tests were used to confirm the compounds' biological activity for antioxidant, antibacterial, antifungal, anticancer, anti-lipase, and α-glycosidase activities. They showed significant activity in most of the tests. The best results were found in DPPH antioxidant, antimicrobial, and α-glycosidase. Overall, the results of these compounds support other researchers to complete studies for further clinical trials and make them as best choices of human drugs.

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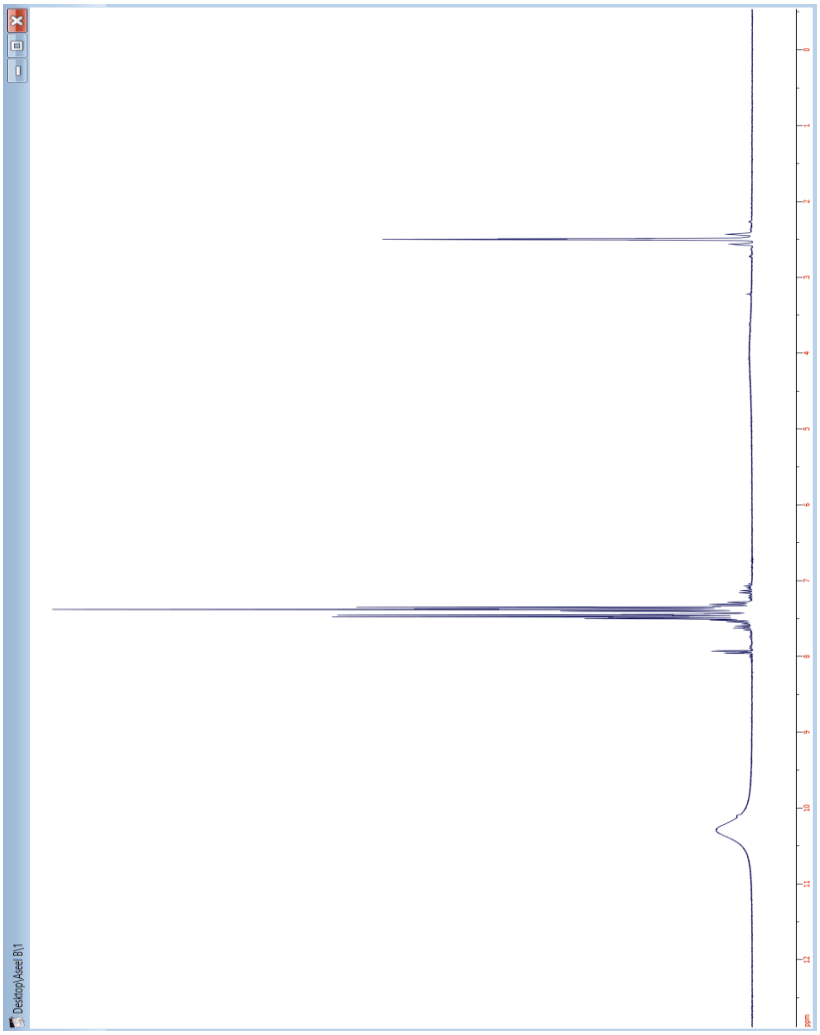
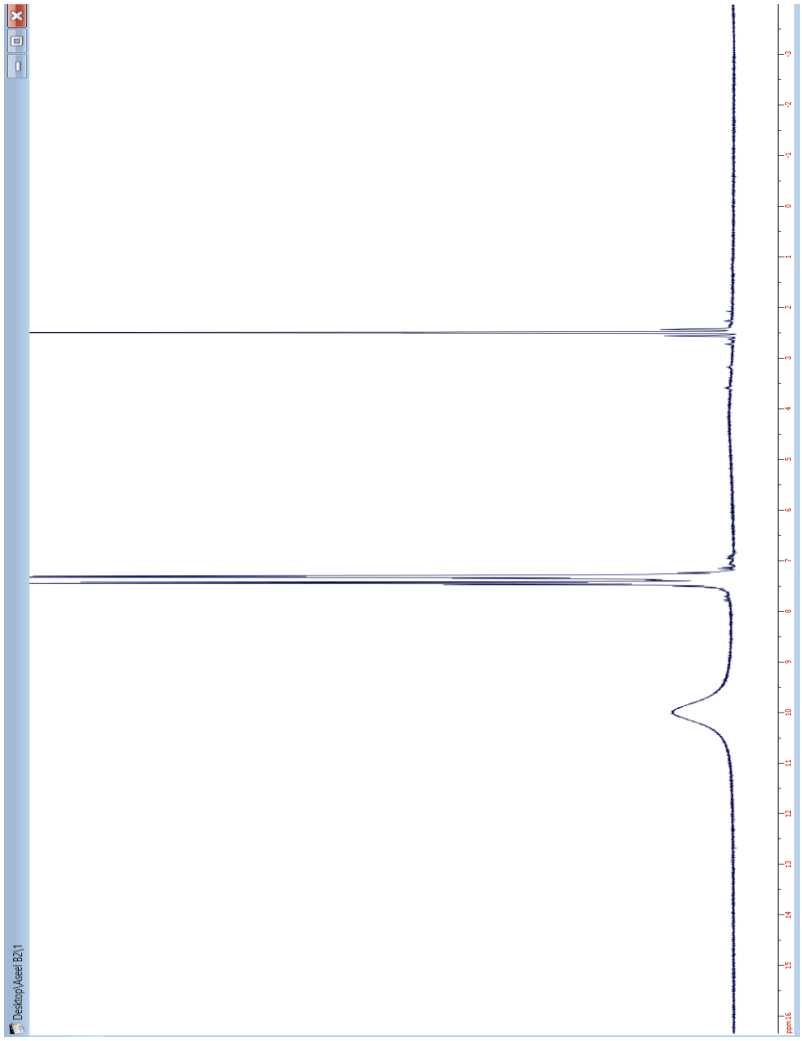
Appendix

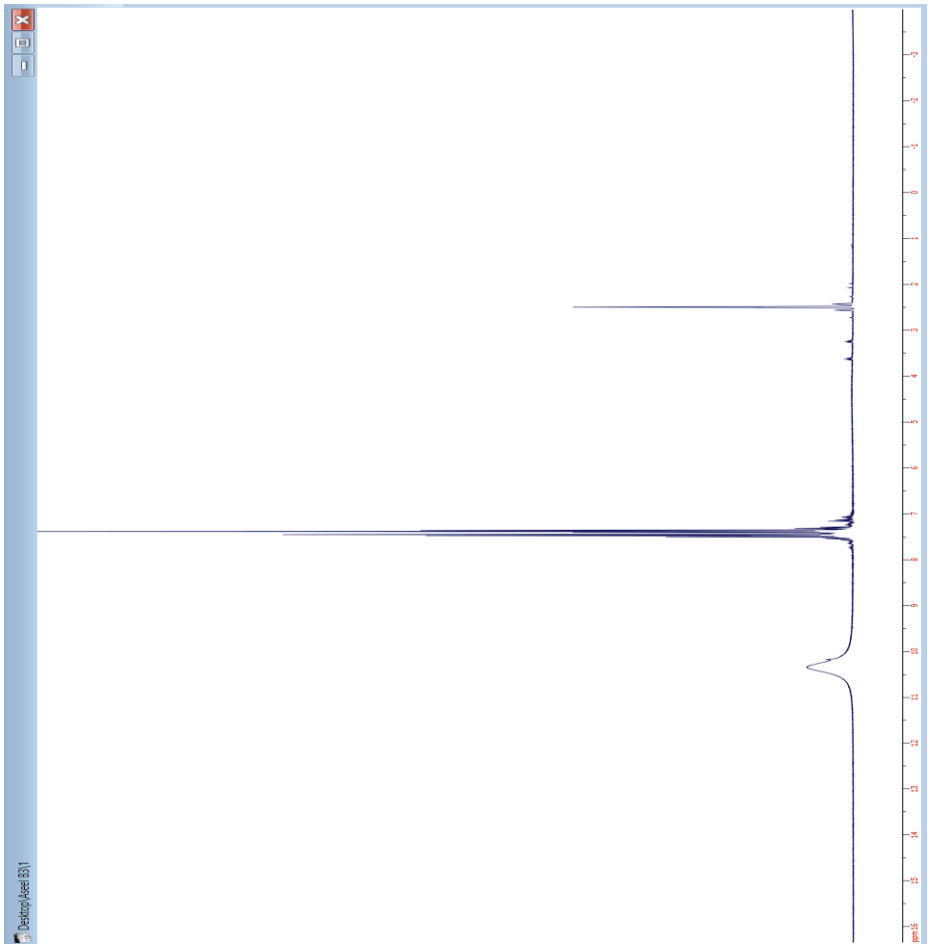
NMR graph for compound I (2-phynlaminethyl benzoic acid)



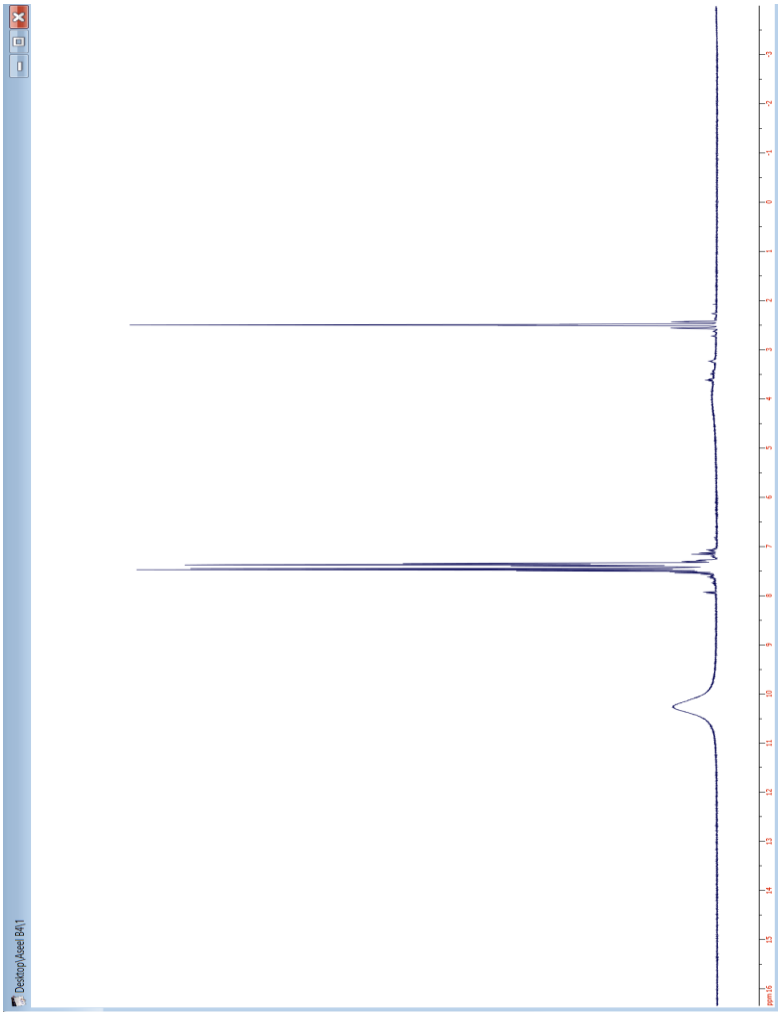
NMR graph for compound II (2-phynlaminethyl-2-hydroxybenzoic acid)



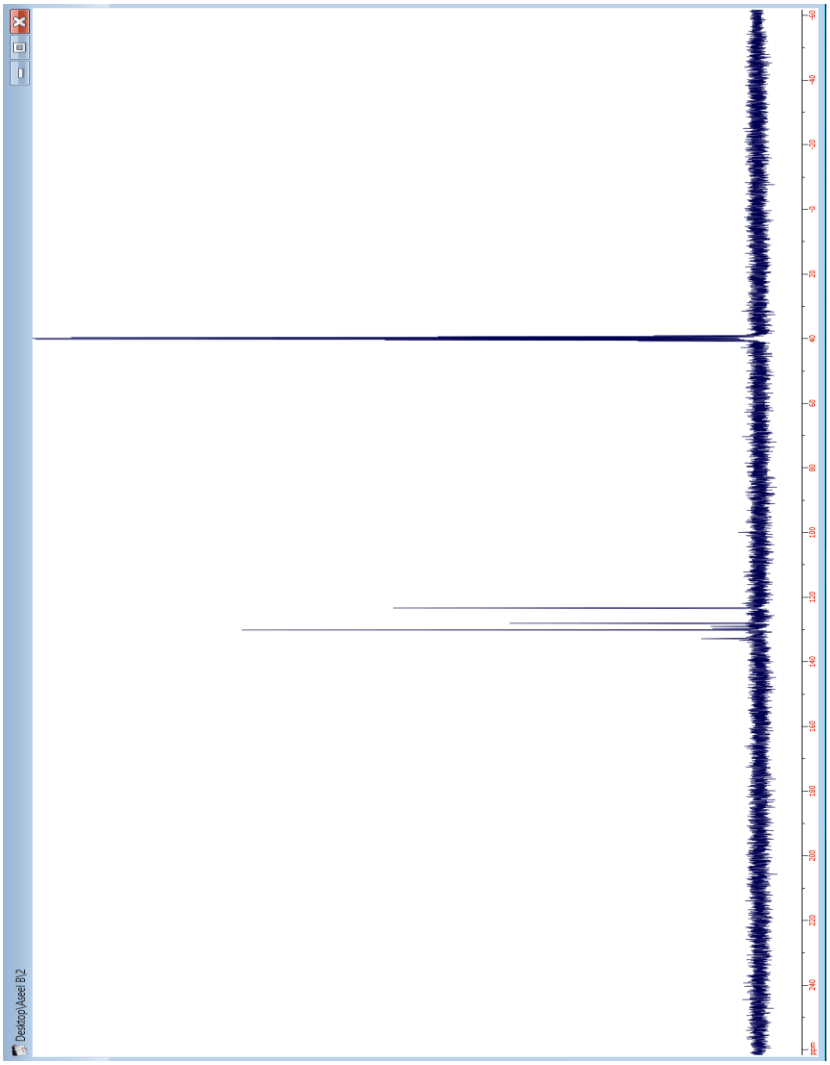
NMR graph for compound III (2-phynlaminethyl-3-hydroxybenzoic acid)



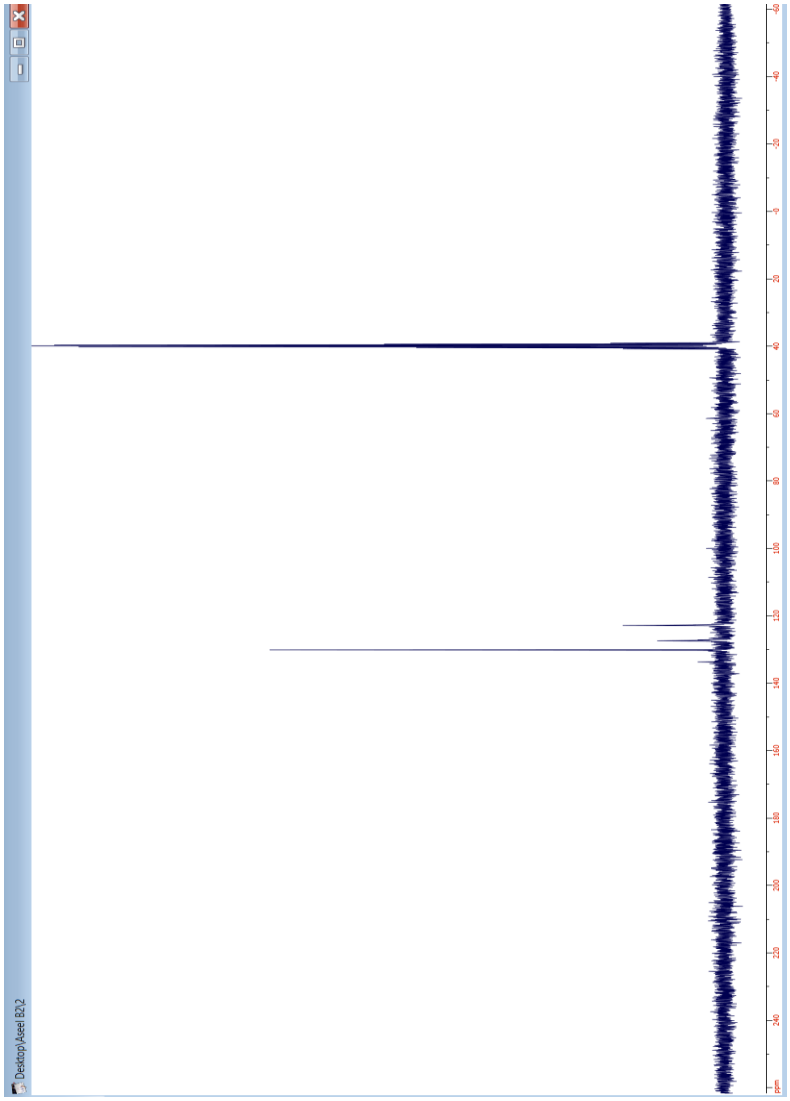
NMR graph for compound IV (2-phynlaminethyl-4-hydroxybenzoic acid)



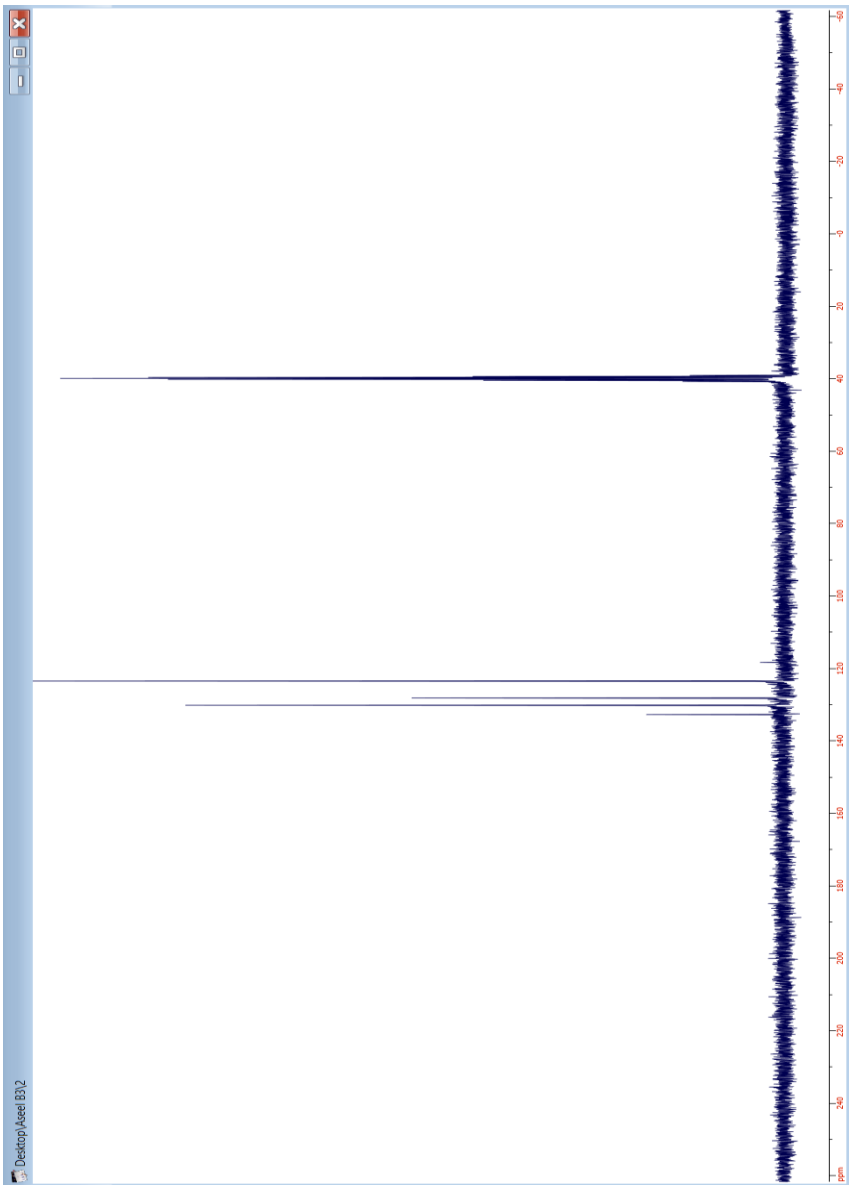
^{13}C graph for compound I (2-phynlaminethyl benzoic acid)



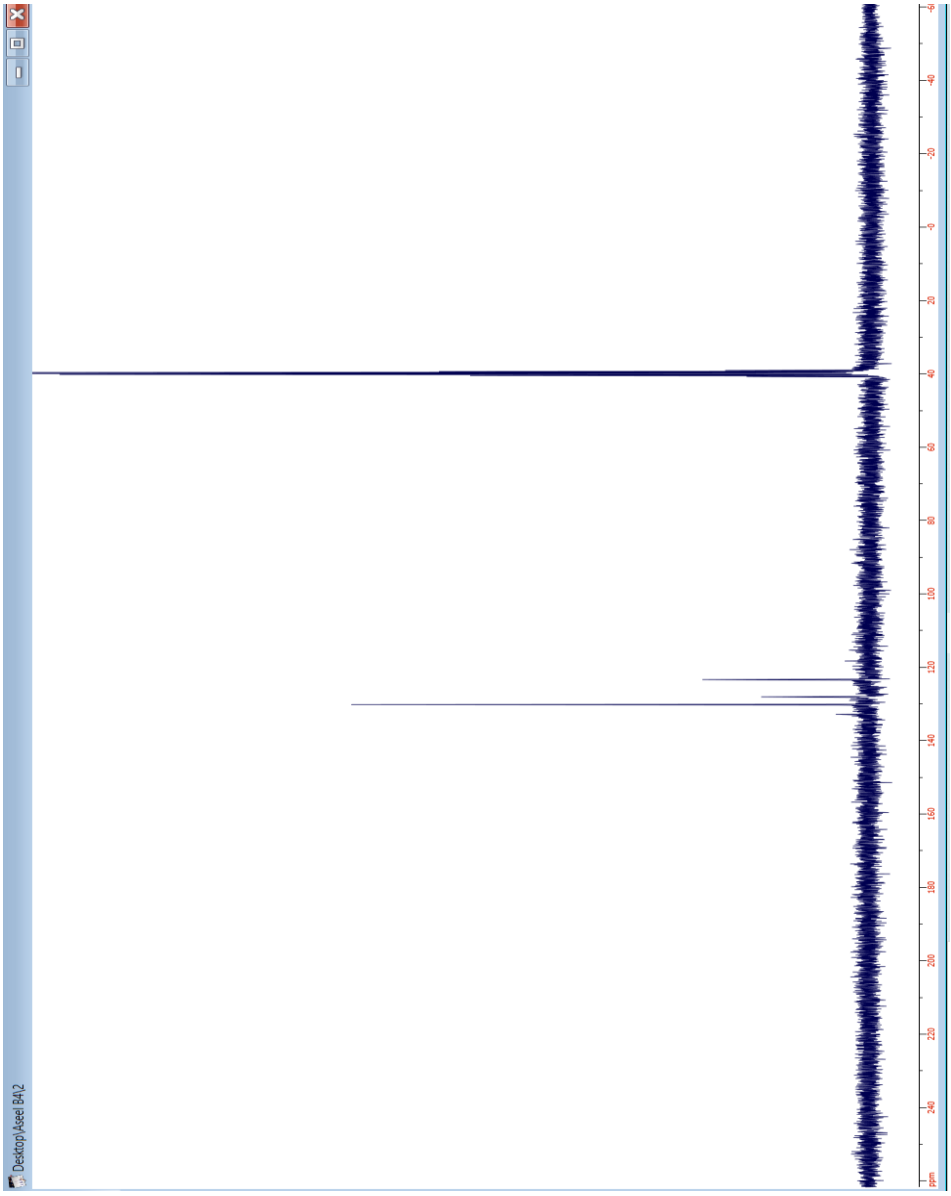
^{13}C graph for compound II (2-phynlaminethyl-2-hydroxybenzoic acid)



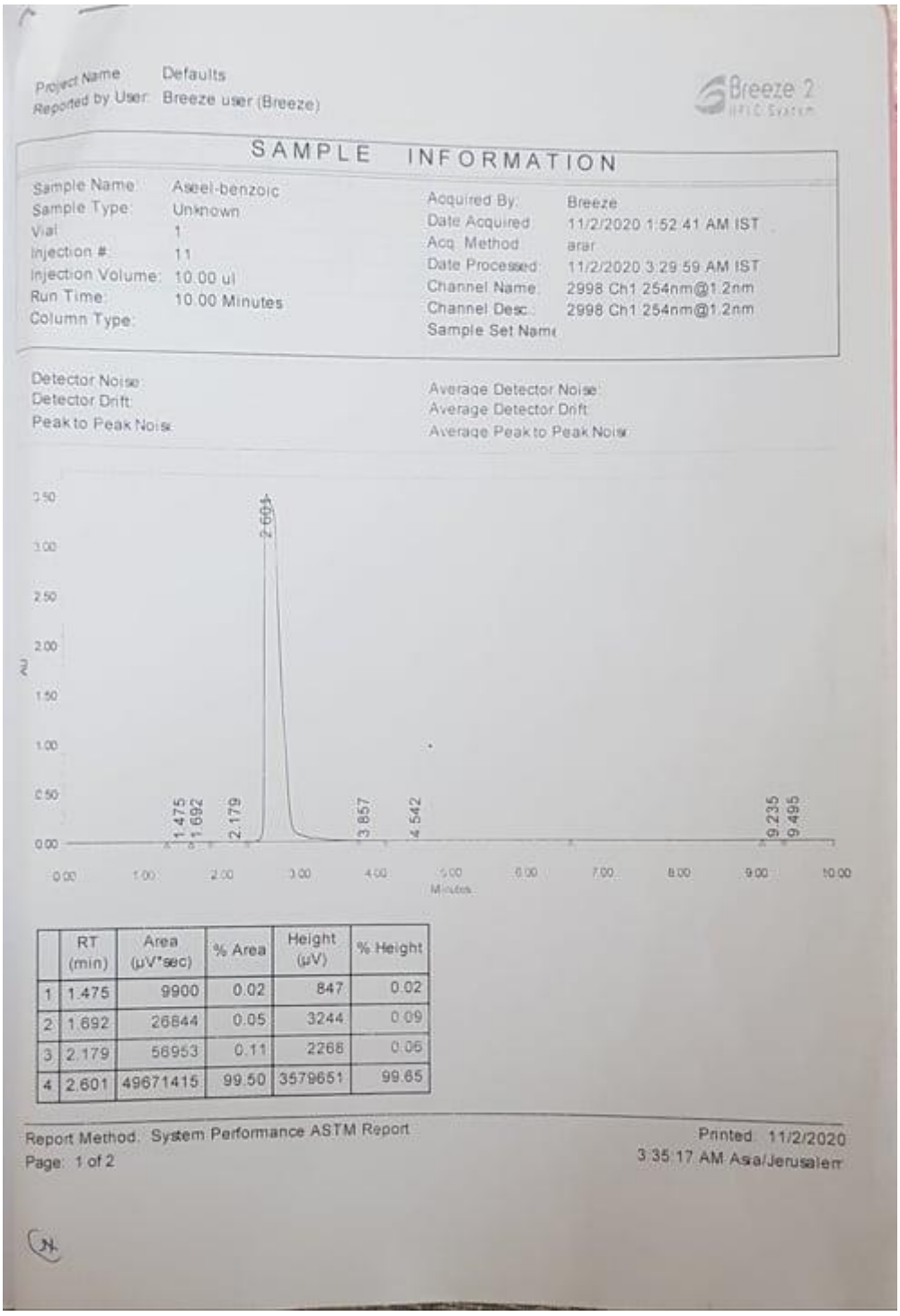
^{13}C graph for compound III (2-phynlaminethyl-3-hydroxybenzoic acid)



^{13}C graph for compound IV (2-phynlaminethyl-4-hydroxybenzoic acid)



HPLC graph for compound I (2-phynlaminethyl benzoic acid)



Project Name: Defaults
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

SAMPLE INFORMATION

Sample Name:	Aseel-benzoic	Acquired By:	Breeze
Sample Type:	Unknown	Date Acquired:	11/2/2020 1:52:41 AM IST
Vial:	1	Acq. Method:	arar
Injection #:	11	Date Processed:	11/2/2020 3:29:59 AM IST
Injection Volume:	10.00 ul	Channel Name:	2998 Ch1 254nm@1.2nm
Run Time:	10.00 Minutes	Channel Desc.:	2998 Ch1 254nm@1.2nm
Column Type:		Sample Set Name:	

Detector Noise:
Detector Drift:
Peak to Peak Noise:

Average Detector Noise:
Average Detector Drift:
Average Peak to Peak Noise:

	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	1.475	9900	0.02	847	0.02
2	1.692	26844	0.05	3244	0.09
3	2.179	56953	0.11	2268	0.06
4	2.601	49671415	99.50	3579651	99.65

Report Method: System Performance ASTM Report
Page: 1 of 2

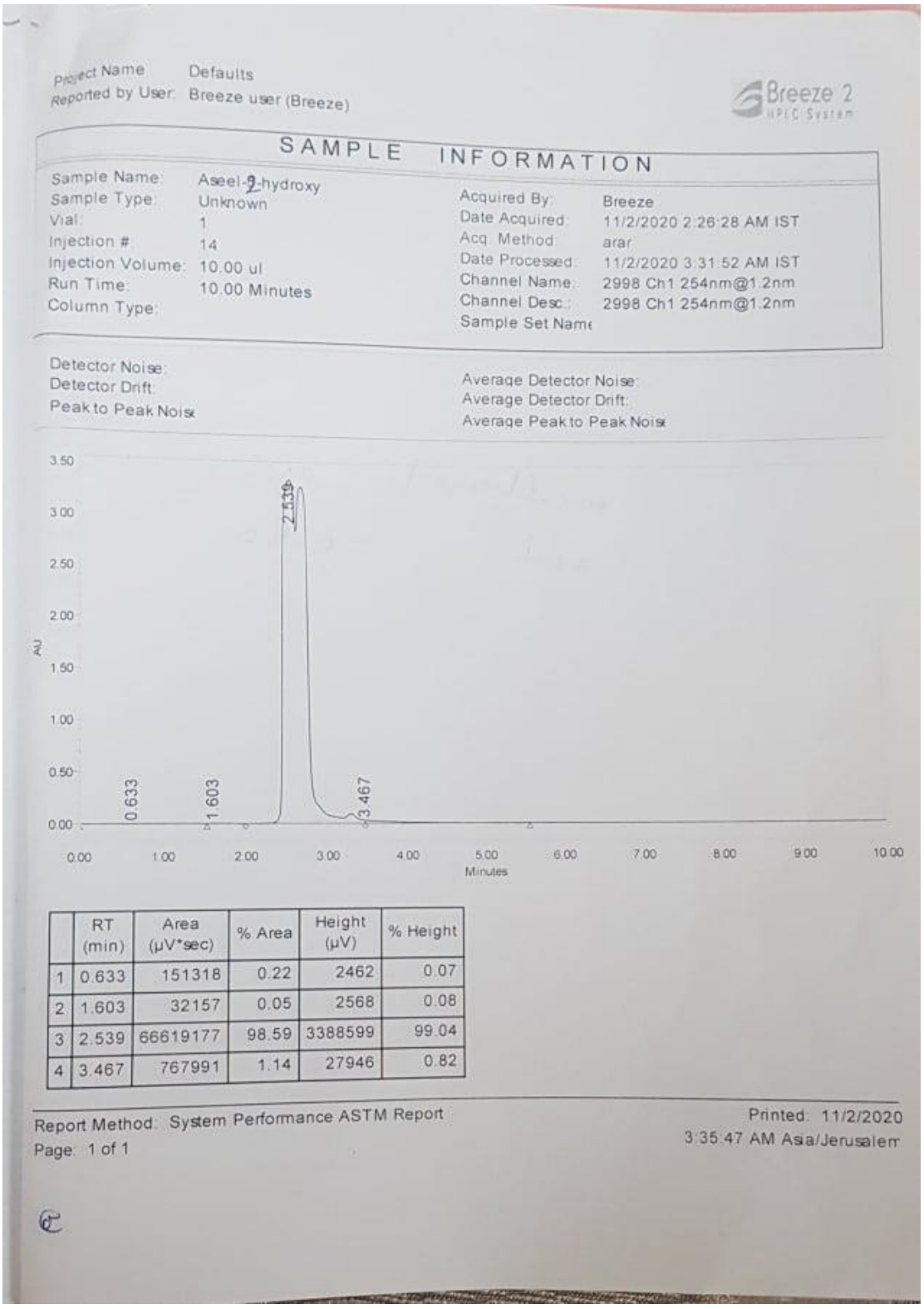
Printed: 11/2/2020
3:35:17 AM Asia/Jerusalem

Project Name Defaults
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
5	3.857	63945	0.13	4901	0.14
6	4.542	88969	0.18	1156	0.03
7	9.235	808	0.00	75	0.00
8	9.495	2239	0.00	99	0.00

HPLC graph for compound II (2-phynlaminethyl-2-hydroxybenzoic acid)



Project Name: Defaults
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

SAMPLE INFORMATION

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Sample Type:	Unknown	Date Acquired:	11/2/2020 2:26:28 AM IST
Vial:	1	Acq. Method:	arar
Injection #:	14	Date Processed:	11/2/2020 3:31:52 AM IST
Injection Volume:	10.00 ul	Channel Name:	2998 Ch1 254nm@1.2nm
Run Time:	10.00 Minutes	Channel Desc.:	2998 Ch1 254nm@1.2nm
Column Type:		Sample Set Name:	

Detector Noise:
Detector Drift:
Peak to Peak Noise:

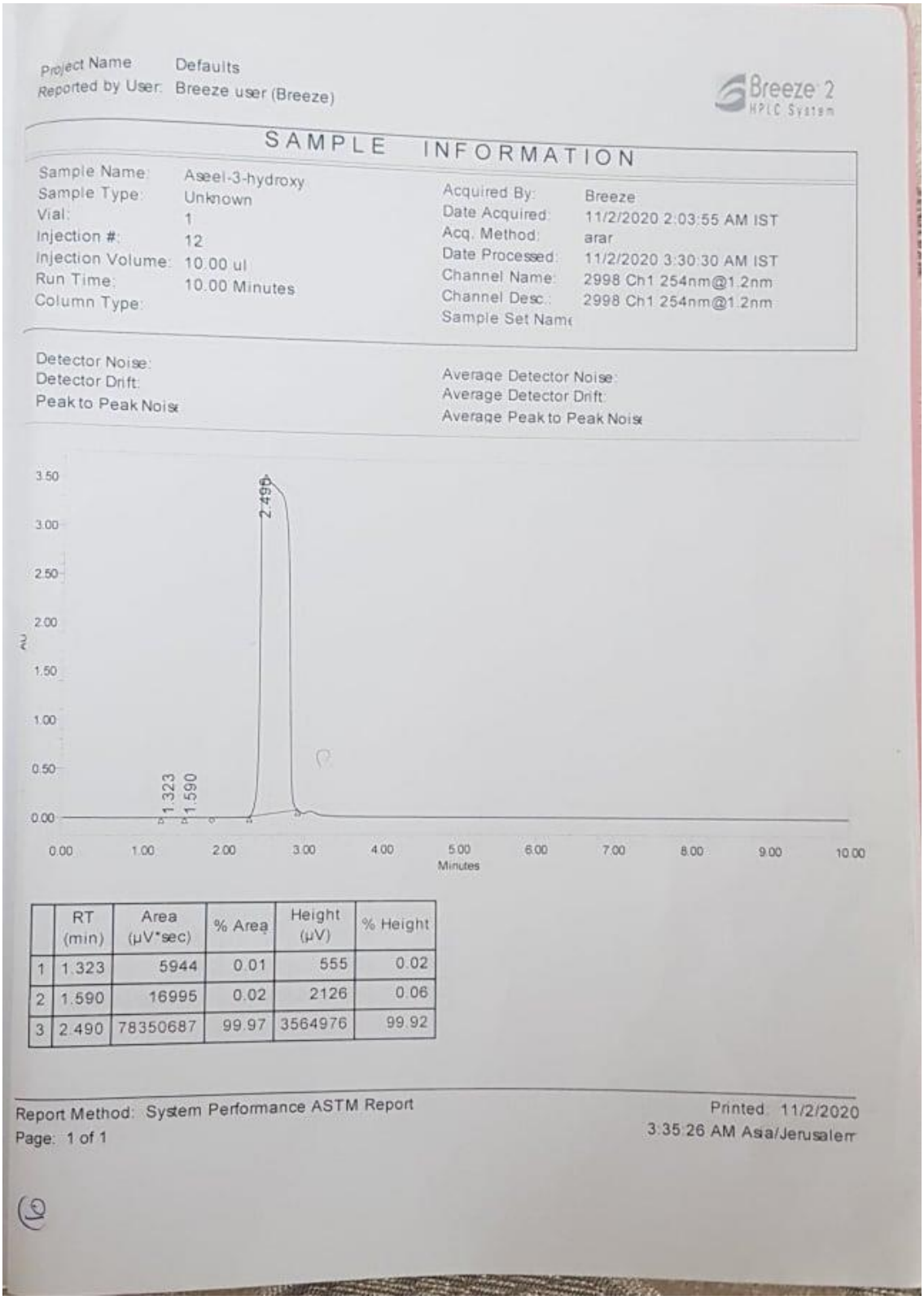
Average Detector Noise:
Average Detector Drift:
Average Peak to Peak Noise:

	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	0.633	151318	0.22	2462	0.07
2	1.603	32157	0.05	2568	0.08
3	2.539	66619177	98.59	3388599	99.04
4	3.467	767991	1.14	27946	0.82

Report Method: System Performance ASTM Report
Page: 1 of 1

Printed: 11/2/2020
3:35:47 AM Asia/Jerusalem

HPLC graph for compound III (2-phynlaminethyl-3-hydroxybenzoic acid)



Project Name: Defaults
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

SAMPLE INFORMATION

Sample Name:	Aseel-3-hydroxy	Acquired By:	Breeze
Sample Type:	Unknown	Date Acquired:	11/2/2020 2:03:55 AM IST
Vial:	1	Acq. Method:	arar
Injection #:	12	Date Processed:	11/2/2020 3:30:30 AM IST
Injection Volume:	10.00 ul	Channel Name:	2998 Ch1 254nm@1.2nm
Run Time:	10.00 Minutes	Channel Desc.:	2998 Ch1 254nm@1.2nm
Column Type:		Sample Set Name:	

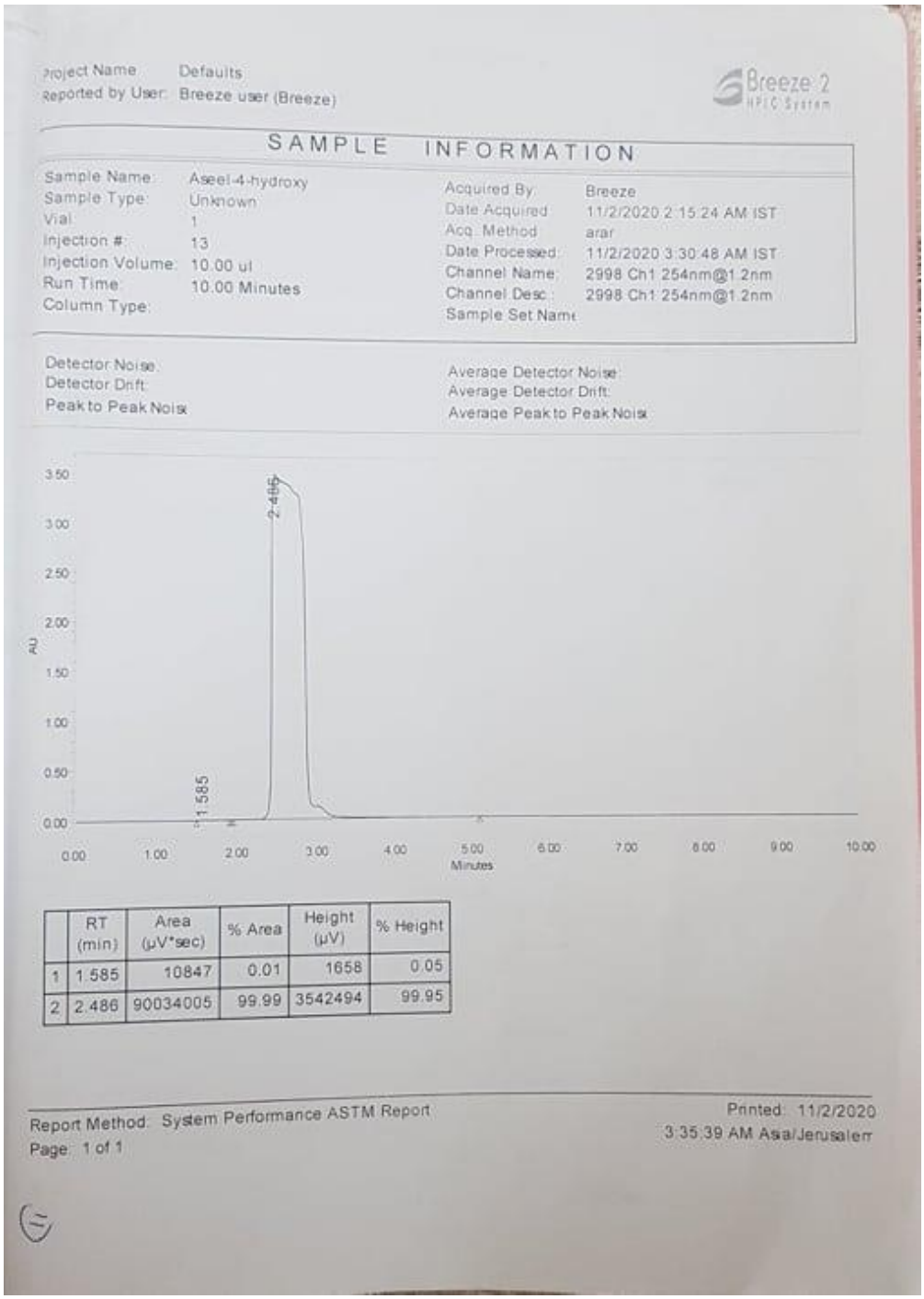
Detector Noise:
Detector Drift:
Peak to Peak Noise:
Average Detector Noise:
Average Detector Drift:
Average Peak to Peak Noise:

	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	1.323	5944	0.01	555	0.02
2	1.590	16995	0.02	2126	0.06
3	2.490	78350687	99.97	3564976	99.92

Report Method: System Performance ASTM Report
Page: 1 of 1

Printed: 11/2/2020
3:35:26 AM Asia/Jerusalem

HPLC graph for compound IV (2-phynlaminethyl-4-hydroxybenzoic acid)



Project Name: Defaults
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

SAMPLE INFORMATION

Sample Name:	Aseel-4-hydroxy	Acquired By:	Breeze
Sample Type:	Unknown	Date Acquired:	11/2/2020 2:15:24 AM IST
Vial:	1	Acq. Method:	arar
Injection #:	13	Date Processed:	11/2/2020 3:30:48 AM IST
Injection Volume:	10.00 ul	Channel Name:	2998 Ch1 254nm@1.2nm
Run Time:	10.00 Minutes	Channel Desc.:	2998 Ch1 254nm@1.2nm
Column Type:		Sample Set Name:	

Detector Noise:
Detector Drift:
Peak to Peak Noise:

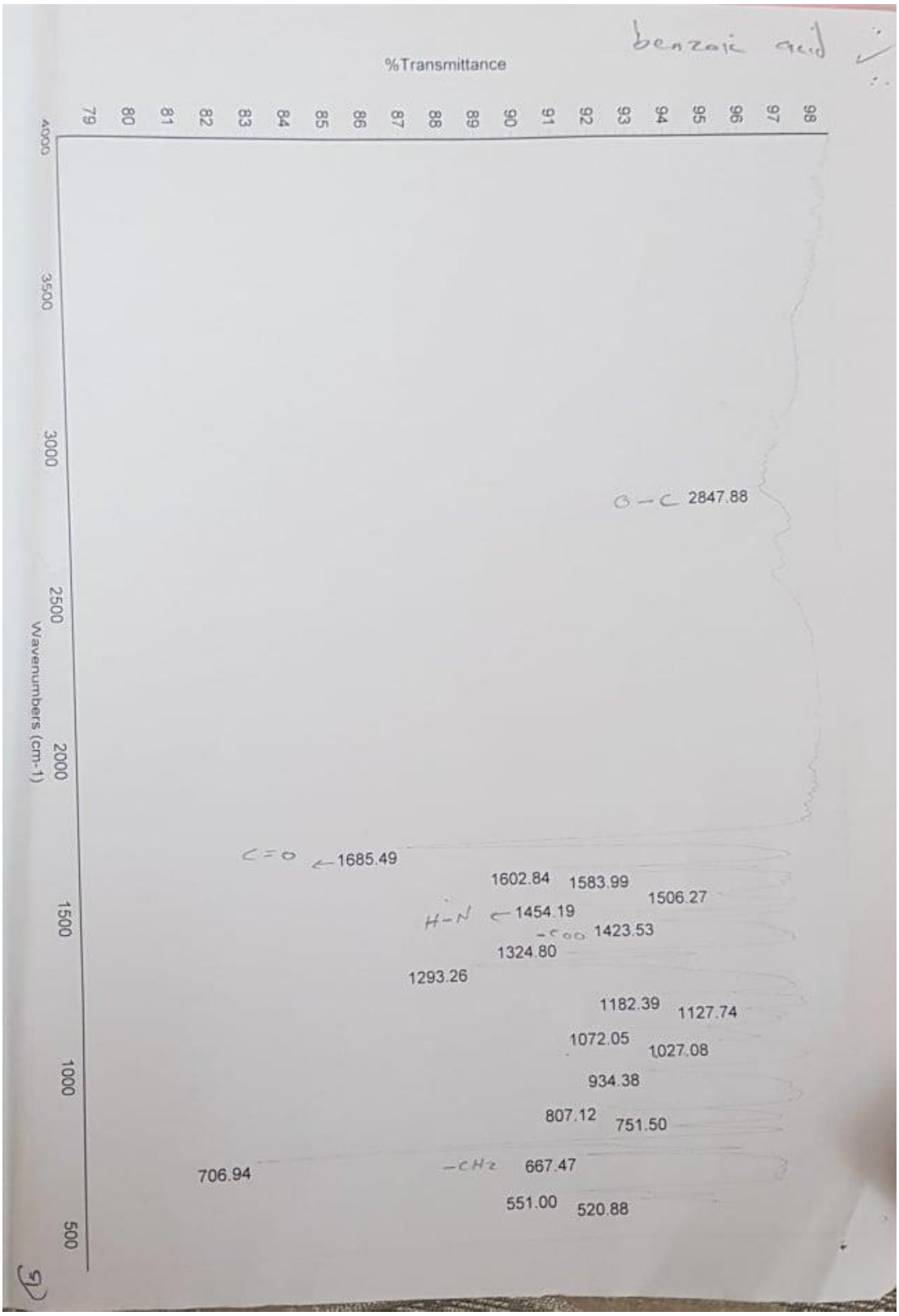
Average Detector Noise:
Average Detector Drift:
Average Peak to Peak Noise:

	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	1.585	10847	0.01	1658	0.05
2	2.486	90034005	99.99	3542494	99.95

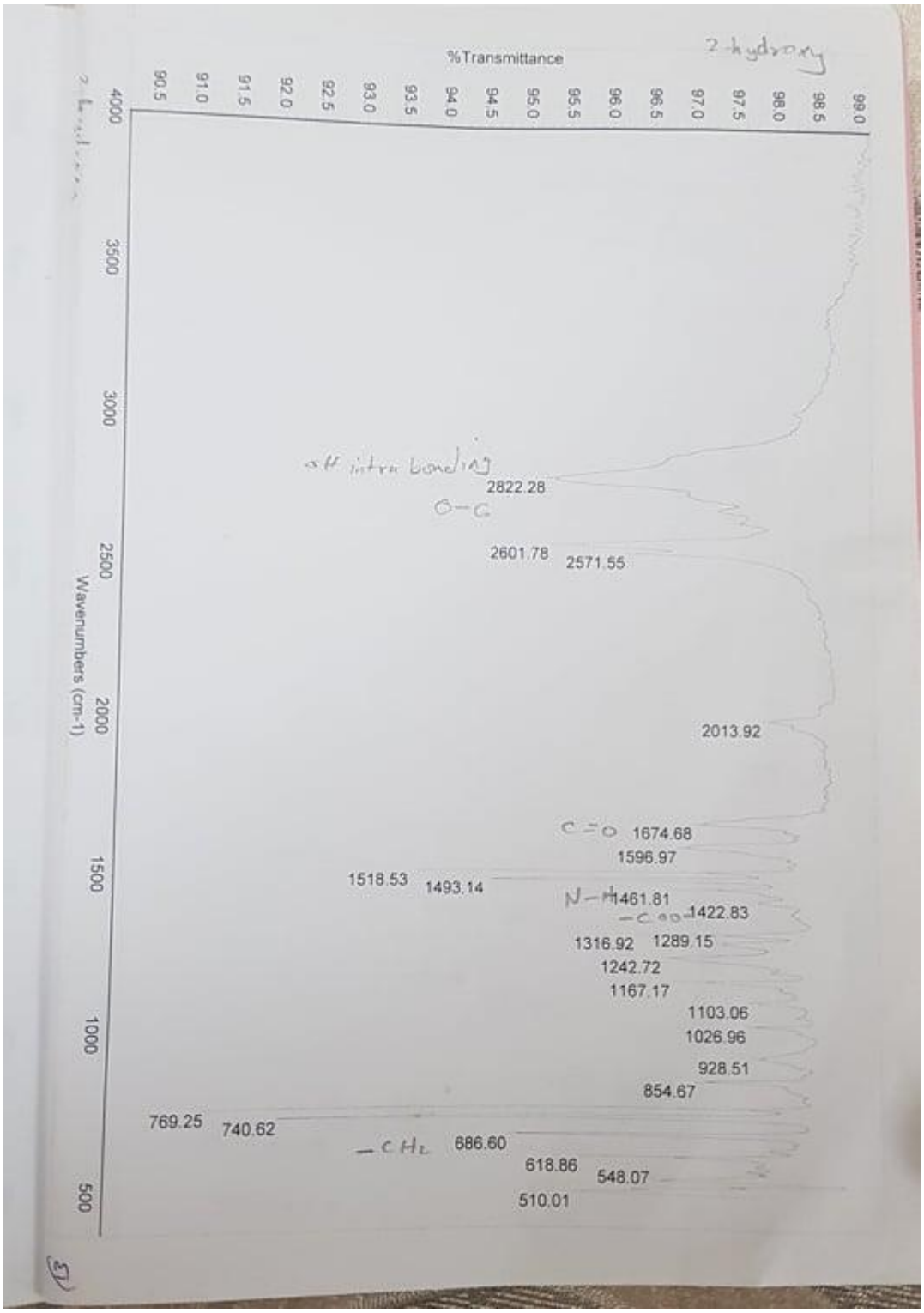
Report Method: System Performance ASTM Report
Page: 1 of 1

Printed: 11/2/2020
3:35:39 AM Asia/Jerusalem

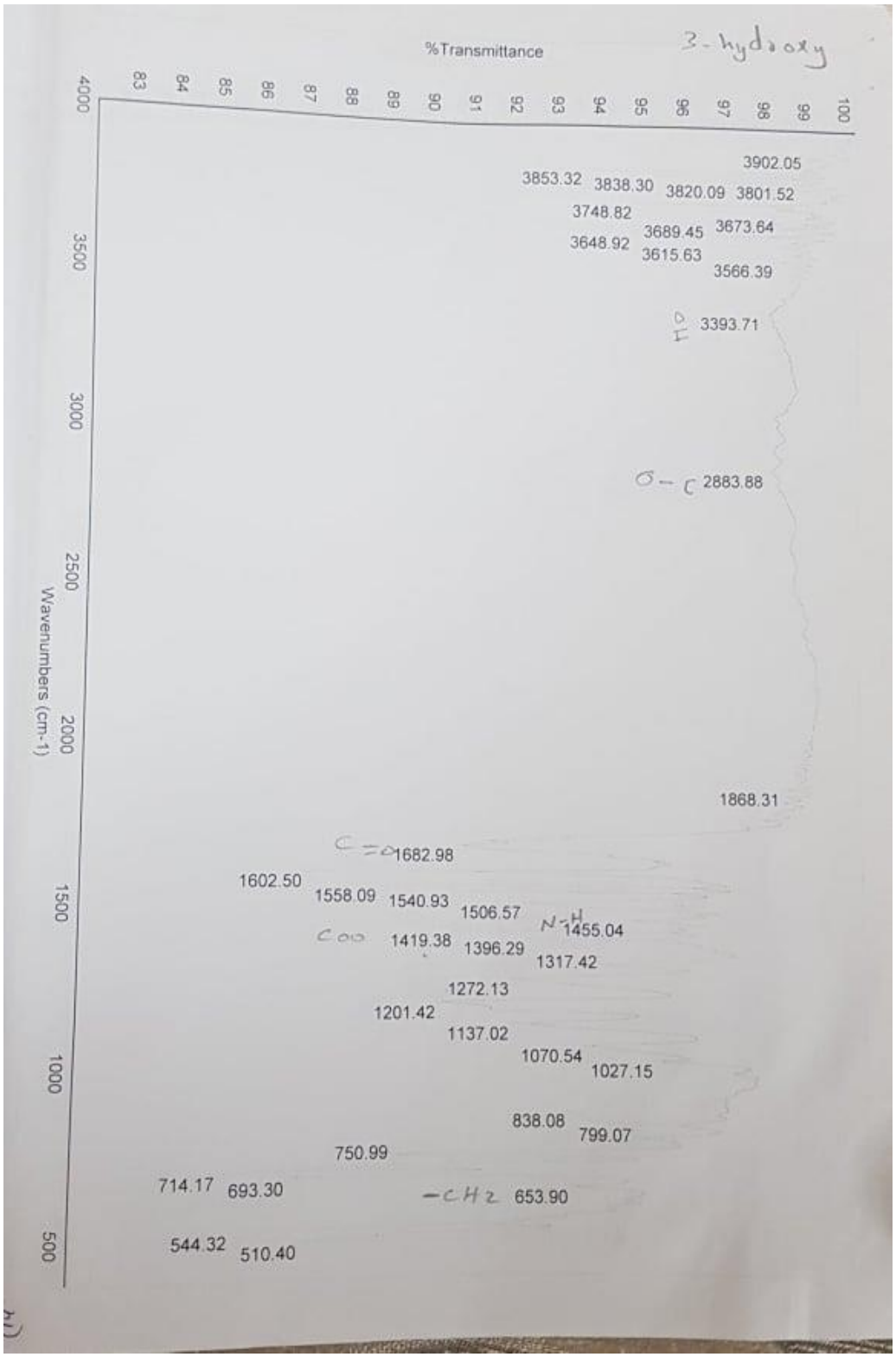
IR graph for compound I (2-phynlaminethyl benzoic acid)



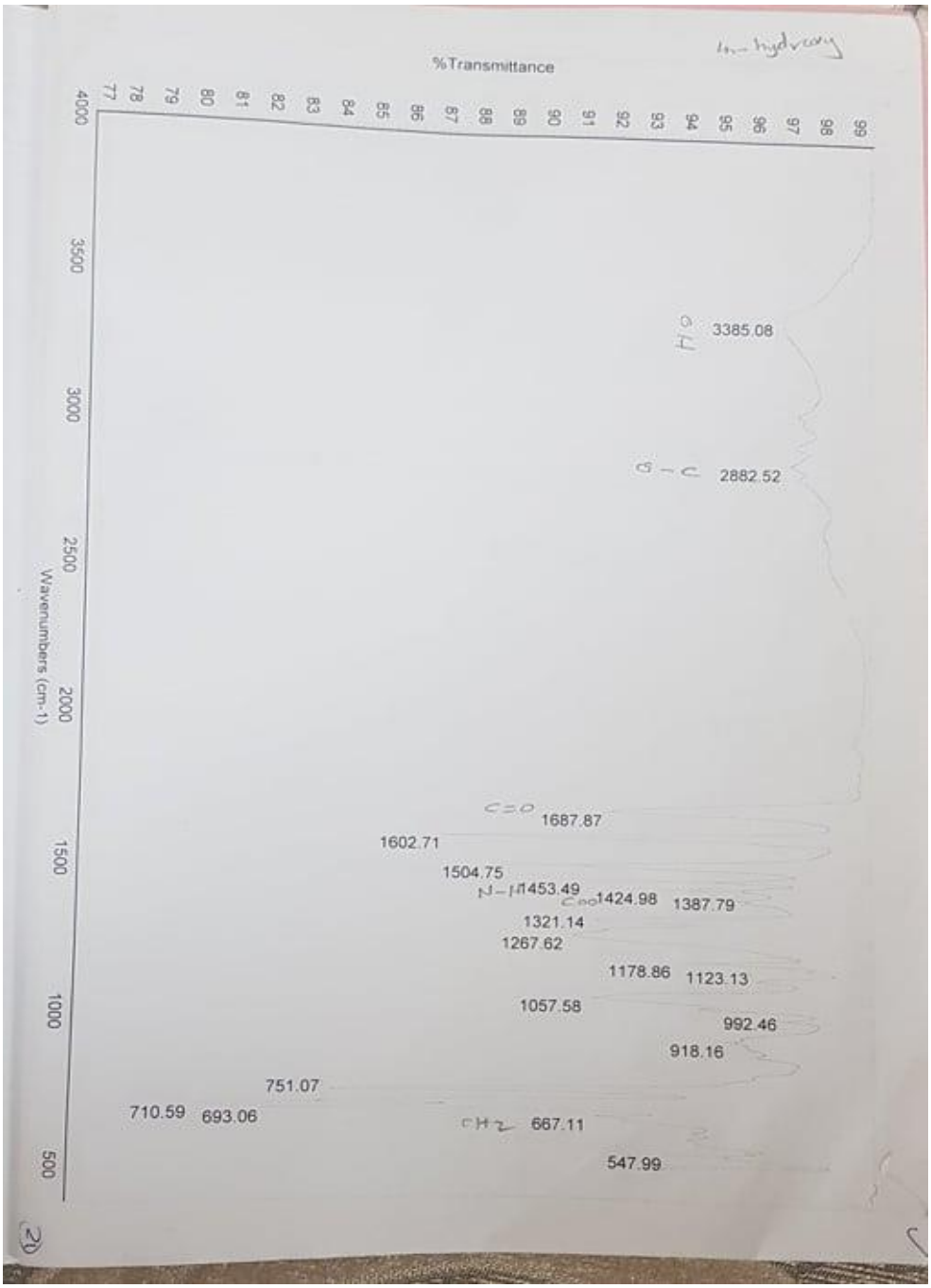
IR graph for compound II (2-phynlaminethyl-2-hydroxybenzoic acid)



IR graph for compound III (2-phynlaminethyl-3-hydroxybenzoic acid)



IR graph for compound IV (2-phynlaminethyl-4-hydroxybenzoic acid)



جامعة النجاح الوطنية

كلية الدراسات العليا

تخليق استرات حمض الأمين العطرية من 2- الفينيل امينيثانول واستكشاف بعض أنشطتها البيولوجية

إعداد

أسيل ماهر حمامرة

إشراف

د. نضال جرادات

د. أحمد خساتي

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم الصيدلانية في كلية الدراسات العليا، جامعة النجاح الوطنية، نابلس، فلسطين.

2021

تخليق استرات حمض الأمين العطرية من 2- الفينيل امينيثانول واستكشاف بعض أنشطتها البيولوجية

إعداد

أسيل ماهر حمامرة

إشراف

د. نضال جرادات

د. أحمد خساتي

الملخص

موضوع هذه الرسالة هو تحضير أربعة استرات حمض أميني عطري من تفاعل حمض البنزويك ومشتقاته وهي (2-هيدروكسي، 3-هيدروكسي، 4-هيدروكسي حمض بنزويك) مع 2-فينيل أمين إيثانول. تم إنشاء هياكل هذه الإسترات الأمينية بواسطة Proton nuclear magnetic resonance (^{1}H-NMR),carbon-13 (C^{13}) nuclear magnetic resonance, and Fourier transforms infrared (FT-IR). تم اختبار استرات الأحماض الأمينية العطرية لأنشطتها المضادة للأكسدة والبكتيريا والفطريات ومضادات السرطان وanti-lipase و-α glycosidase. كان نشاط المركبات كمضادات للأكسدة في DPPH حوالي (IC_{50} > 0.5 mg / ml)، بينما كانت قيمة Trolox IC_{50} = 0.1 mg / ml.كما تم اختبار المركبات من حيث نشاطها المضاد للبكتيريا والفطريات ضد: (المكورات العنقودية الذهبية، الاشريكية القولونية، البروتيوس الشائع، كليبسيلا الالتهاب الرئوي، الزائفة العصبية، المبيضات البيضاء، والمعوية المكورات العنقودية الذهبية) وأظهرت قيمة MIC (4 mg/ml). بينما لم تظهر النتائج نشاطًا كبيرًا تجاه الخلايا السرطانية. علاوة على ذلك، بالنسبة anti-lipase (IC_{50} = 231 مجم / مل) وIC_{50} = 87.5 مجم / مل لـ α-glycosidase وهو نفس قيمة الأكاربوز .أظهرت المركبات العطرية المُصنّعة نشاطًا مهمًا في معظم الاختبارات. كانت أفضل النتائج في الأنشطة المثبطة لمضادات الأكسدة DPPH ومضادات الميكروبات و α-glycosidase.

تم تحضير المركبات الاصطناعية بكفاءة عن طريق تفاعل استرات الحمض و2- فينيل امينيثانول. تم تأكيد هوية المركب باستخدام طريقة التحليل الطيفي مثل HNmr1 وUV و13 carbon وIR. تم تحديد مركبات إستر الأحماض الأمينية اعتمادًا على بعض الخصائص الفيزيائية مثل كروماتوغرافيا الطبقة الرقيقة ونقاط الانصهار. تم استخدام العديد من الاختبارات لتأكيد النشاط البيولوجي للمركبات لأنشطة مضادات الأكسدة ومضادات الجراثيم والفطريات ومضادات السرطان ومضادات الليباز وα-glycosidase. أظهروا نشاطًا كبيرًا في معظم الاختبارات. تم العثور على أفضل النتائج في مضادات الأكسدة DPPH ومضادات الميكروبات وα-glycosidase. بشكل عام، تدعم نتائج هذه المركبات الباحثين الآخرين لإكمال الدراسات لإجراء مزيد من التجارب السريرية وجعلها أفضل الخيارات للعقاقير البشرية.