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THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY**

**Isolation and characterization of pectin and natural product
from passion fruits rind (*Passiflora edulis* flavicarpa)**



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ABBREVIATIONS AND SYMBOLS

%.....	percentage
mg.....	milligram
ml.....	milliliter
gm.....	gram
N.....	Normality
Temp.....	Temperature
TLC.....	Thin Layer Chromatography
UV lamp.....	Ultra Violet lamp
UV-VIS.....	Ultra Violet- visible
NMR.....	Nuclear Magnetic Resonance
IR.....	Infrared
HPLC.....	High Performance Liquid Chromatography
H ₂ SO ₄	Sulfuric acid
NaOH.....	Sodium hydroxide
MeOH.....	Methanol
CHCl ₃	Chloroform
HCl.....	Hydrochloric Acid
EtoAc.....	Ethylacetate
1D.....	One Dimension
DE.....	Degree of Esterification
MeOH.....	Methoxyl Content
AUA.....	AnhydroUronic Acid Content
Fig.....	Figure
ASTU.....	Adama Science and Technology University

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ABSTRACT

*Passion fruit rind is reported to be a good source of pectin and other natural products but it is not well known in Ethiopia. Pectin, a naturally occurring polysaccharide, has in recent years gained increasingly in importance. The benefits of natural pectin are more and more appreciated by scientists and consumer due to its biodegradability. Pectin is the methylated ester of polygalacturonic acid. It is commercially extracted from citrus peels and apple pomace under mildly acidic conditions. The pectin, by itself or by its gelling properties, was employed in pharmaceutical industry, health promotion and treatment. Pectins have been utilized for their functionality in foods for many years. Ubiquitous in the conservation and preservation industries, development of pectin has centered on its use to impart texture in high sugar systems. This research dealt with optimization of pectin extraction from yellow passion fruit (*Passiflora edulis*) rind by varying the type of acids for extraction, pH, temperature and time of extraction. Further, the extracted pectin was characterized using quantitative analysis to determine and compare the equivalent weight, methoxyl and AUA content, and DE. The overall result showed that the two pectin extracts were different and passion fruit rinds studied was sources of low-ester pectin. In addition, a grounded passion fruit rind was extracted by using organic solvents and the extract was subjected to repeated chromatography such as TLC, CC and PTLC by different solvent. This led to isolation of a new compound from the chloroform extract, PE-3, whose structure was elucidated as 5-cyclohexyl-6-hydroxyoctyl ethyl phthalate. The structure of the compounds was determined using spectroscopy techniques including UV-VIS, IR and 1D NMR.*

Chapter 1

1.0. Introduction

1.1. Background

Passion fruit belongs to the *Passifloraceae* family featuring over 600 species worldwide. Among these, the fruits of only about 20 varieties are edible and the most cultivated specie in the world is the yellow passion fruit (*Passiflora edulis flavicarpa*) [1].

Passion fruit is a high acid food (pH 3.2) due to the predominance of two acids, citric (93-96% of total) and malic (3-6% of total) acids. Passion fruit also contains about 14.45g sugar/100g of edible portion, including fructose, glucose and sucrose, along with seven others in trace amounts. The acids and sugars add to the unique taste and serve as a preservative for the tropical fruit [2].

Earlier studies that reported the chemical composition of passion fruit rind concluded that the rind of the fruit contain a higher nutrient than the pulp. Moreover, the rinds are a good source of fiber and pectin [3].

Flavonoids, mainly C-glycosylflavones, are the major constituents of *P. edulis* leaves and pulp and a HPLC method was reported for the quantification of iso-orientin (**10**) and total flavonoids in *P. edulis* pulp, suggested that *P. edulis* fruits may be comparable with other flavonoid food sources such as orange juice or sugarcane juice [4].

The main raw materials used to produce commercial pectin are apple pomace and citrus peels. However, pectin may be obtained from yellow passion fruit peel (*Passiflora edulis flavicarpa*) rind, after the fruit is used for production of juice. The peel represents about half of the fruit mass [5]. Pectin is a high-value functional food ingredient widely used as gelling agents e.g. jam, jelly, emulsion stabilizer and thickener in sauces, it can also be used in medicine as a fiber source, a supplement to lower cholesterol and increase immunity or as dentistry adhesive [6,7].

The process for production of commercial pectin is not released in its entirety by the industries. It is well known the influence of various factors on extraction such as acid type, temperature, acid concentration and solvent characteristics of raw material, among others [8]. Usually the pectin are extracted from the peel of citrus fruit and apple with strong mineral acids such as

phosphoric, sulfuric, nitric or hydrochloric acid at pH range 1-3 at temperatures between 80 and 100°C [9].

The use of strong acids for extraction of pectin has some disadvantages such as environmental pollution and equipment corrosion. The replacement of extractor agents commonly used by weak organic acids can help to minimize adverse effects. Studies have reported the use of citric acid as extracting agent very efficiently [9].

1.2. Statement problem

The peel of passion fruit in juice processing represents about half of the fruit mass. Because of significant juice production, the peels, as a major waste, have become a substantial burden to the environment. Hence it is necessary to find a feasible way to turn the peels into useful products or to adequately dispose of them, seeking a positive environmental impact. It is reported that passion fruit rind extract have medicinal value but in Ethiopia passion fruit rind has hitherto never been investigated.

1.3. Justification of the study

Passion fruit rind is a known source of pectin and other natural products. Pectin, a naturally occurring polysaccharide, has in recent years gained increasingly in importance. The benefits of natural pectin are also more and more appreciated by scientists and consumer due to its biodegradability. Pectin is the methylated ester of polygalacturonic acid. It is commercially extracted from citrus peels and apple pomace under mildly acidic conditions. The pectin, by itself or by its gelling properties, was employed in pharmaceutical industry, health promotion and treatment. Pectin's have been utilized for their functionality in foods for many years. Ubiquitous in the conserves and preserves industries, development of pectin's has centered on its use to impart texture in high sugar systems. Studies reported that extraction of pectin from passion fruit rind has high economic value. That's why this study was done to manufacture pectin from passion fruit rinds in Ethiopia in feasible way.

1.4. Significant of the study

The passion fruit rind in Africa juice Tibila factory is a waste material and they take the waste to the farm and use it as organic fertilizer, but on other hand this waste material is the source of pectin and other products which is used as a food ingredient, widely used as gelling agent and stabilizer and also used in medicine as a fiber source, a supplement to lower cholesterol and increase immunity, as dentistry adhesive. This study contributes to future production of pectin and other natural products from *P. edulis* rind in Ethiopia.

2. Objective of the research

2.1. General objective

- To investigate the chemical composition of *P. edulis* rind.

2.2. Specific objective

- To investigate the extractability and composition of pectin from passion fruit rind with citric acid and sulfuric acid using the different variables (pH, temperature and extraction time).
- To characterize pectin from passion fruit rind.
- To extract and isolate natural products from passion fruit rind.
- To elucidate the structures of the isolated compounds using spectroscopic methods

Chapter 3

3.0. Literature review

3.1. Distribution of Passion fruit

Passion fruit belongs to the Passifloraceae family featuring over 600 species worldwide. Among these, the fruits of only about 20 varieties are edible and the most cultivated species in the world is the yellow passion fruit (*Passiflora edulis*) [1].

A native to South America, from Paraguay, Brazil and Argentina, Passion Fruit is (*Passiflora* species) a choice crop of the tropical and sub-tropical belt because it is one of the few fruits that comes into production within a year and is referred to as the ‘fruit cash crop’. It yields a sprightly juice rich in vitamins A, B₅ and C [3].

While the origin of the passion fruit plant is unknown, it is generally believed to be native of Brazil, which is the world’s largest producer and consumer of fresh and processed yellow passion fruit, accounting for 50-60 % of the total world production. Today passion fruit grows nearly everywhere in the tropical belt, but South America is the main exporter of yellow passion fruit concentrated juice as its demand is growing worldwide [5]. Also, the *Passiflora* species are widely used as phytotherapeutic agents in Brazil [6].

3.2. Phytochemicals present in passion fruit

There are three primary groups of active chemicals in passion fruit: alkaloids, glycosides and flavonoids. A large amount of variability is noticed with regards to the incidence and quantity of particular phytochemical within the same species of passion fruit. There is a lot of evidence that the passion fruit could be a powerful medicinal source but much more research needs to be done to unlock these potentially potent remedies [2].

3.2.1. Alkaloids

Harmala alkaloids are a group of β -carboline compounds. Harmala alkaloids (1) present in passion fruit include harmane, harmine (2), harmol (3), harmalol. The amount of harmala

alkaloids present in the purple passion fruit is 0.012% and in the yellow passion fruit 0.700%. Others include theobromine while passiflorin is an alkaloid glycoside [2].

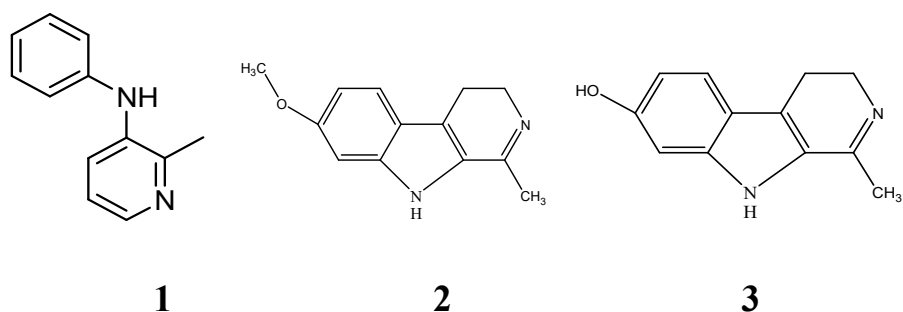


Fig 1: Structure of representative harmala alkaloids isolated from *P. edulis*

3.2.2. Flavonoids

The amount of flavonoids present in the purple passion fruit variety is 1.060% and in the yellow passion fruit variety 1.000%. Studies have indicated that in order for the passion fruits sedative effect to exist the glycosides and flavonoids must be combined because individually they produce opposite effects [2].

Rutin, quercetin and kaempferol belong to the flavonol (4) subgroup of flavonoids. Catechin (5) and epicatechin belong to the flavan-3-ols subgroup of flavonoids. Cyanidin-3-glucoside belong to the anthocyanidin subgroup of flavonoids. Luteolin (6) and apigenin both belong to the flavone and benzoflavone subgroup of flavonoids. Flavone glycosides present in passion fruit include homoorientin, isoorientin (10), orientin, isovitexin, vitexen, iso-schaftoside, schaftoside, saponaretin, saponarin and various other glycosides [2].

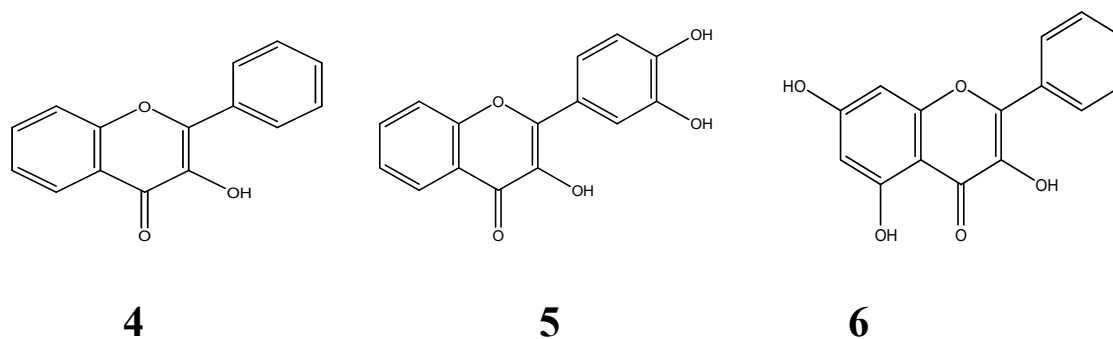


Fig 2: Structure of some flavonoids isolated from *P. edulis*

3.2.3. Carotenoids

The amount of carotenoids present in the purple passion fruit variety is 1.160% and in the yellow passion fruit variety is 0.058%. Carotenoids include β -cryptoxanthin, prolycopene, *cis*- γ -carotene (9), α -caroene (8), β -carotene, 13-*cis*- β -carotene (7), neurosporene and γ -carotene and α -carotene. In one study γ -carotene and β -carotene were present in the largest amounts in the yellow passion fruit. In another study, yellow passion fruit was found to be rich resource of α -carotene and β -carotene when compared to other tropical fruits but not in comparison to tomatoes and/or carrots [2].

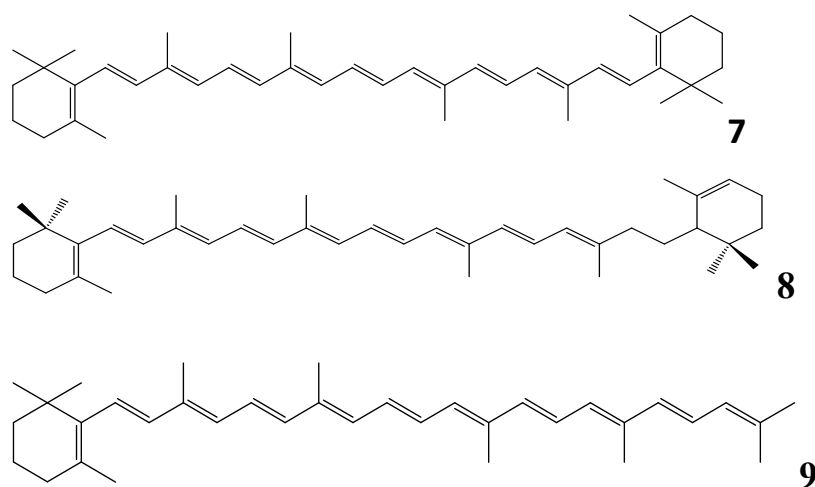


Fig 4. Structure of some carotenoids isolated from *P. edulis*

3.3.4. Bioactivity

Passion fruit proved to have analgesic (pain-reliever) anti-anxiety, anti-inflammatory, antispasmodic, cough suppressant, aphrodisiac, central nervous system depressant, diuretic, hypotensive (lowers blood pressure) and sedative activities. Besides, it is traditionally reported to possess anticonvulsant, antidepressant, astringent, cardiogenic (tones, balances, strengthens the heart), disinfectant, nervine (balances/calms nerves), neurasthenic (reduce nerve pain), tranquilizer and vermifuge (expels worms) activities. It may have promising and powerful effects on neurological disorders and chronic diseases such as heart disease and cancer [2].

3.3. Passion fruit rind

P. edulis is native to Brazil, the world's largest producer of this species. In Brazil, where the species is known popularly due to its acidity as “sour maracujá” (“maracujáazedo”), or simply “maracujá”, it is widely cultivated and used in the food industry (processed juices). Passion fruit rinds represent about half of the fruit's mass, and because of the high volume of juice production, the rinds represent a major waste product and a substantial burden to the environment [10].

The full data obtained herein and in a complementary study of the antioxidant activity on stimulated neutrophils and myeloperoxidase activity, suggest a direct correlation between antioxidant capacity and isoorientin (**8**) content in *P. edulis* rinds. The healthy rind extracts presented higher DPPH•scavenging ability than PWV-infected rinds, and also showed the highest isoorientin content, probably partially responsible for the scavenging capacity of the extracts [10].

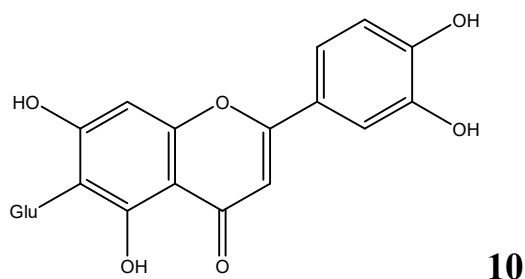


Fig 4: Structure of compound isolated from *P. edulis* rinds by HPLC

Polyphenols, including flavonoids, provide beneficial health effects that are attributed to their antioxidant and chelating abilities. Therefore, it is important to study natural sources of flavonoids and evaluate their activity to assess their potential as possible functional foods [11].

The antioxidant capacity of passion fruit rinds (healthy and infected with PWV) was found to be considerably higher than in other foods such as sugarcane juice ($EC_{50} = 100.80 \pm 2.56 \text{ g L}^{-1}$) and passion fruit pulp ($EC_{50} = 38.50 \pm 2.28 \text{ g L}^{-1}$) [12].

3.4. Pectin

Pectin substances are a large family of structural elements of primary cell walls and intercellular regions of higher plants where they function as hydrating agent and cementing material of the cellulosic network. They are commonly produced during the initial stages of primary cell growth and make about one –third of the cell-wall dry substances. The highest concentration of pectins in the cell wall is seen in the middle lamella, with a gradual decrease from the primary cell wall toward the plasma membrane [13].

3.5. Source and production of pectin

Pectin is a complex mixture of polysaccharides that makes up about one third of the cell wall dry substance of higher plants. Much smaller proportions of these substances are found in the cell walls of grasses. The highest concentrations of pectin are found in the middle lamella of cell wall, with a gradual decrease as one passes through the primary wall toward the plasma membrane [14]. Although pectin occurs commonly in most of the plant tissues, the number of sources that may be used for the commercial manufacture of pectin is very limited. Because the ability of pectin to form gel depends on the molecular size and degree of esterification (DE), the pectin from different sources does not have the same gelling ability due to variations in these parameters. Therefore, detection of a large quantity of pectin in a fruit alone is not in itself enough to qualify that fruit as a source of commercial pectin [15]. At present, commercial pectins are almost exclusively derived from citrus peel or apple pomace, both by-products from juice (or cider) manufacturing. Apple pomace contains 10-15% of pectin on a dry matter basis. Citrus peel contains of 20-30% [16]. From an application point of view, citrus and apple pectins are largely equivalent. Citrus pectins are light cream or light tan in colour; apple pectins are often darker.

Alternative sources include sugar beet waste from sugar manufacturing, sunflower heads (seeds used for edible oil), passion fruit and mango waste [17]. Commercially, pectin is extracted by treating the raw material with hot dilute mineral acid at pH about 2. The precise length of extraction time varies with raw material, the type of pectin desired, and from one manufacturer to another. The hot pectin extract is separated from the solid residue as efficiently as possible.

This is not easy since the solids are by now soft and the liquid phase are viscous. The viscosity increases with pectin concentration and molecular weight. There is a compromise between efficient extraction and solids separation and operating cost. The pectin extract may be further clarified by filtration through a filter aid. The clarified extract is then concentrated under vacuum. Powdered pectin can be produced by mixing the concentrated liquid from either apple or citrus with an alcohol (usually isopropanol and ethanol). The pectin is separated as a stringy gelatinous mass, which is pressed and washed to remove the mother liquor, dried and ground. This process yields pectin of around 70% esterification (or methoxylation). To produce other types, some of the ester groups must be hydrolysed. This is commonly carried out by the action of acid, either before or during a prolonged extraction, in the concentrated liquid, or in alcoholic slurry before separation and drying. This process can produce a range of calcium reactive low methoxylpectins. Hydrolysis using ammonia results in the conversion of some of the ester groups into amide groups, producing 'amidated low methoxylpectins' [16].

3.6. Chemical properties of pectin

Chemically, pectins are a family of complex heteropolysaccharides comprised by a diversity of carbohydrate residues. Like most other plant polysaccharides, pectin are polydisperse in composition and molecular size, that is, they are heterogeneous with respect to both chemical structure and molecular weight. Their composition varies with the source and conditions of extraction, location, and other environmental factors [18].

Pectin is a polymer of α -galacturonic acid with a variable number of methyl ester groups. However, pectin also contains α -L-rhamnopyranosyl residues in the backbone chain and branch chains of arabinan and galactan, and their fine structure vary considerably [19]. Some of the carboxylic groups of galacturonic acid molecules in the pectin chains are methyl esterified and the percentage of esterified groups is expressed as DE (degree of esterification). Depending on the degree of esterification, pectin is divided into two major groups: high-ester pectin, with DE higher than 50%, and low-ester pectin, with DE lower than 50% [20].

In high-ester pectin, the junction zones are formed by the cross-linking of homogalacturan through hydrogen bond and the hydrophobic interaction between methoxyl groups, both of

which are promoted by high-sugar concentration and low pH. In low-ester pectin, junction zones are formed by calcium cross-linking between free carboxyl groups [21].

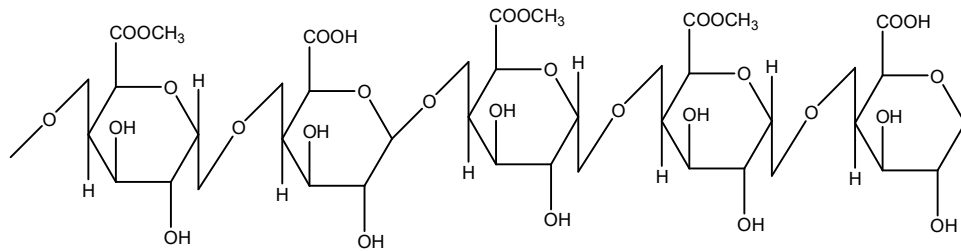


Fig 5: Structure of pectin

3.7. Uses of pectin

Only a few source materials have been used for commercial production of pectin as food additive. One of the reasons for this is that most of the pectin materials present in nature does not have any functional properties; in particular, the ability to form sugar acid gel systems and this property has been the main requirement of commercial pectin until recently [22].

Chapter 4

4. Materials and Methods

4.1. Apparatus and Instruments

Hot air oven, mortar and pestle, electrical grinder, conical flask, digital beam balance, P^H meters, water bath, glass rod, refrigerator, measuring cylinder, Whatman no. 1 filter paper, round bottom flask, rotary evaporator, TLC plat, TLC chamber, column, silica gel (60-120 mesh size), UV lamp, GENESY'S 2PC UV-Vis scanning spectrometer (200-800nm), Bruker Advance 400MHz spectrometer in CDCl₃ (¹H-NMR: 400 MHz, ¹³C-NMR: 100.60 MHz), Infrared spectrawere obtained on Perkin-Elmer BX infrared spectrometer (400-4000cm⁻¹) using KBr.

4.2. Chemicals and reagents

Citric acid, sulfuric acid, distilled water, 99.9% ethanol, sodium hydroxide, hydrochloric acid, phenolphthalein red, sodium chloride, methanol, chloroform, ethyl acetate, n-hexane.

4.3. Plant material collection and preparation

Passion fruits rind was collected from the Africa JUICETibila S.C juice factory located in Ethiopia, Oromia region. The raw material was prepared for the experiments in the following way: The rind of passion fruit was washed and dried in an air-forced oven at 55 °C until constant weight. Then the dried passion fruit peels were ground by mortar and pestle as well as with electrical grinder to a dry 60-sieve-size powder. The ground powders were packaged in polyethylene bags for the experiment.

4.4. Pectin extraction

The extraction procedure was based on the protocol of [18], considering several variables. 150ml distilled water was transferred in 250ml conical flask and 45g, 15g, and, 10 g citric acid was added to get 1.5, 2.0 and 2.5 pH solutions respectively and 125ml distilled water was transferred in 250ml conical flask and added 0.6ml, 1.4ml and 2.3ml of H₂SO₄ was added to get 1.5, 2.0 and 2.5 pH solutions respectively. Then 5g of powdered passion fruit rind sample was added in to the prepared solution. Each of solution was boiled in water bath at 60°C, 80°C, and 100°C for 30min, 45min and 60min separately with continuous stirring. Then the hot acid extract was filtered

through the ordinary screen with 1-mm mesh size equipped with two-layer cheesecloth, and the filtrate was cooled down in 4⁰C refrigerator (Table 1 and 2).

4.5. Centrifugation and purification of pectin

The filtrate solution was coagulated using volume of 150ml of 96% ethanol for citric acid extract and 125ml of 96% ethanol for coagulate and left for 3h in refrigerator. Then the coagulated pectin was separated by filtration, washed once with of 70% acidic ethanol (0.5% HCl), then with 70% ethanol to a neutral pH and finally with 96% ethanol. The resulting material was air dried.

The pectin yield was calculated using the following equation:

$$Yield = \frac{\text{dried pectin (g)}}{\text{initial dry passion fruit rind (g)}} \times 100\% \dots \dots \dots (1)$$



Fig 6: Pectin extract with citric acid (a) and with sulfuric acid (b)

4.6. Characterization of Pectin

4.6.1. Equivalent Weight

Before analysis, pectin was finely ground with mortar and pestle.

Equivalent weight was determined following Ranganna's method (1995). 0.5 g sample was taken in a 250 ml conical flask and 5 ml ethanol was added. 1 g of sodium chloride to sharpen the end point and 100 ml of distilled water was added. Finally 6 drops of phenolphthalein indicator was added and titrated against 0.1 N NaOH. Titration point was indicated by purple color. This neutralized solution was stored for determination of methoxyl content. The equivalent weight was calculated by the following equation:

$$\text{Equivalent weight} = \frac{\text{wt. of sample}}{v \text{ (ml) of alkali} \times \text{normality of alkali}} \times 1000 \dots \dots \dots (3)$$

4.6.1. Methoxyl Content (MeO)

Determination of MeO was done by using the Ranganna's method (Mohamed & Hasan, 1995). The neutral solution was collected from determination of equivalent weight, and 25 ml of sodium hydroxide (0.25 N) was added. The mixed solution was stirred thoroughly and kept at room temperature for 30 min. After 30 min 25 ml of 0.25 N HCl was added and titrated against 0.1 N NaOH to the same end point as before like in equivalent weight titration.

The methoxyl content was calculated the following equation:

$$\text{Methoxyl Content\%} = \frac{\text{ml of alkali} \times \text{normality of alkali} \times 3.1}{\text{Wt. of sample}} \dots \dots \dots (4)$$

4.6.2. Total Anhydrouronic Acid Content (AUA)

Total AUA of pectin was obtained by the following formula (Mohamed & Hasan, 1995).

$$\% \text{AUA} = \frac{176 \times 0.1z \times 100}{W \times 1000} + \frac{176 \times 0.1y \times 100}{W \times 1000} \dots \dots \dots (5)$$

Where molecular unit of AUA (1 unit) = 176 g

Where, z = volume (ml) of NaOH from equivalent weight determination.

y = volume (ml) of NaOH from methoxyl content determination.

w = weight of sample

4.6.3. Determination of Degree of Esterification (DE)

The DE of pectin was measured on the basis methoxyl and AUA content (Owens et al., 1952) and was calculated by following formula

$$\%DE = \frac{176 \times \%MeO + 31 \times \%AUA}{100} \times 100 \quad (6)$$

Where $\%MeO$ = Methoxyl content,

$\%AUA$ = Anhydrouronic Acid Content

4.7. Extraction and isolation of natural product

4.7.1. Extraction of natural product

The powdered sample 200g of the passion fruit rind was soaked with n-Hexane for 72hr and then the extract was filtered by Whatman No1 filter paper and concentrated under reduced pressure at a temperature of 40°C using Rota vapor yielded 2.3 g. The marc obtained from n-Hexane extract was further extracted with chloroform for 72hr, filtered by Whatman No1 filter paper and then concentrated under reduced pressure at a temperature of 45°C using Rota vapor yielded 1.0g. The marc obtained from chloroform extract was further extracted with Ethyl acetate for 72hr and then the extract was filtered by Whatman No1 filter paper and concentrated under reduced pressure at a temperature of 45°C using Rota vapor yielded 0.3g. Then the whole 3 concentrated crude extracts were transferred in vessel and kept in refrigerator for further analysis.

The Chloroform crude extract was analyzed using TLC chloroform/ methanol with ratio 100%, 90:10, 80:20, 70:30, 60:40 and 50:50 solvent system as shown in fig 5.



Fig 7: TLC analysis of chloroform crud extract with different ratio of chloroform/Methanol

4.7.1. Isolation of crud extract

The chloroform crud extract (1.0 g) was subjected to silica gel column chromatography (50 g silica gel) and eluted with increasing gradient of methanol in chloroform. A total of 16 fraction were collected and analyzed by TLC.



Fig 8: Column chromatography analyses of chloroform crude extract with chloroform/methanol solvent system

Fraction 7 (0.3 g) was applied on silica gel column chromatography (50g of silica gel) eluted with increasing gradient methanol in chloroform. Fraction 3 was concentrated on rotatory evaporator resulted 15.2mg of compound PE3.

Chapter 5

4.0. Result and Discussion

5.1. Pectin Yield

Pectin was extract from passion fruit rind by the method of acid-base extraction.

The pectin yield from passion fruit peel using citric acid was in the range between 27.27% - 81.08%. At pH 1.5, 60⁰C and 60min the pectin yield was 81.08% and at pH 2.5, 60⁰C and 45min the pectin yield was 27.27% (Table 1).

Table 1. Extraction of Pectin using citric acid

No	Acid	pH	Temp (⁰ C)	Time (min)	Dried pectin(g)	%yield
1.	Citric acid	1.5±0.1	60 ⁰ C	30min	2.58	51.74
2.	Citric acid	1.5±0.1	60 ⁰ C	45min	3.45	69.11
3.	Citric acid	1.5±0.1	60 ⁰ C	60min	4.05	81.08
4.	Citric acid	1.5±0.1	80 ⁰ C	30min	3.92	78.51
5.	Citric acid	1.5±0.1	80 ⁰ C	45min	3.64	72.97
6.	Citric acid	1.5±0.1	80 ⁰ C	60min	3.88	77.71
7.	Citric acid	1.5±0.1	100 ⁰ C	30min	3.69	73.97
8.	Citric acid	1.5±0.1	100 ⁰ C	45min	3.42	68.47
9.	Citric acid	1.5±0.1	100 ⁰ C	60min	3.57	71.38
10.	Citric acid	2.0±0.2	60 ⁰ C	30min	3.34	66.91
11.	Citric acid	2.0±0.2	60 ⁰ C	45min	3.58	71.56
12.	Citric acid	2.0±0.2	60 ⁰ C	60min	3.62	72.49
13.	Citric acid	2.0±0.2	80 ⁰ C	30min	2.25	45.09
14.	Citric acid	2.0±0.2	80 ⁰ C	45min	2.31	46.32
15.	Citric acid	2.0±0.2	80 ⁰ C	60min	2.11	42.23
16.	Citric acid	2.0±0.2	100 ⁰ C	30min	3.25	65.08
17.	Citric acid	2.0±0.2	100 ⁰ C	45min	3.13	62.67
18.	Citric acid	2.0±0.2	100 ⁰ C	60min	3.56	71.26
19.	Citric acid	2.5±0.1	60 ⁰ C	30min	3.53	70.66
20.	Citric acid	2.5±0.1	60 ⁰ C	45min	1.36	27.26
21.	Citric acid	2.5±0.1	60 ⁰ C	60min	1.59	31.93
22.	Citric acid	2.5±0.1	80 ⁰ C	30min	2.42	48.44
23.	Citric acid	2.5±0.1	80 ⁰ C	45min	2.52	50.46
24.	Citric acid	2.5±0.1	80 ⁰ C	60min	3.12	62.42
25.	Citric acid	2.5±0.1	100 ⁰ C	30min	3.46	69.33
26.	Citric acid	2.5±0.1	100 ⁰ C	45min	3.54	70.89
27.	Citric acid	2.5±0.1	100 ⁰ C	60min	3.56	71.31

The pectin yield from passion fruit peel using sulfuric acid was in the range between 15.1%-43.05%. At pH 2.0, 80⁰C and 60min the pectin yield was 43.052% and at pH 1.5, 60⁰C and 30 min the pectin yield was 15.11% (Table 2).

Table 2. Extraction of Pectin using sulfuric acid

No	Acid	pH	Temp (⁰ C)	Time (min)	Dried pectin(g)	%yield
1.	Sulfuric acid	1.5±0.1	60 ⁰ C	30min	0.75	15.11
2.	Sulfuric acid	1.5±0.1	60 ⁰ C	45min	1.00	20.14
3.	Sulfuric acid	1.5±0.1	60 ⁰ C	60min	0.98	19.70
4.	Sulfuric acid	1.5±0.1	80 ⁰ C	30min	1.37	27.49
5.	Sulfuric acid	1.5±0.1	80 ⁰ C	45min	1.32	26.51
6.	Sulfuric acid	1.5±0.1	80 ⁰ C	60min	1.11	22.26
7.	Sulfuric acid	1.5±0.1	100 ⁰ C	30min	1.23	24.71
8.	Sulfuric acid	1.5±0.1	100 ⁰ C	45min	1.65	33.06
9.	Sulfuric acid	1.5±0.1	100 ⁰ C	60min	1.43	28.65
10.	Sulfuric acid	2.0±0.2	60 ⁰ C	30min	2.00	40.02
11.	Sulfuric acid	2.0±0.2	60 ⁰ C	45min	1.85	37.13
12.	Sulfuric acid	2.0±0.2	60 ⁰ C	60min	1.88	37.73
13.	Sulfuric acid	2.0±0.2	80 ⁰ C	30min	1.81	36.25
14.	Sulfuric acid	2.0±0.2	80 ⁰ C	45min	1.85	37.13
15.	Sulfuric acid	2.0±0.2	80 ⁰ C	60min	2.15	43.05
16.	Sulfuric acid	2.0±0.2	100 ⁰ C	30min	1.95	39.14
17.	Sulfuric acid	2.0±0.2	100 ⁰ C	45min	2.02	40.44
18.	Sulfuric acid	2.0±0.2	100 ⁰ C	60min	2.09	41.99
19.	Sulfuric acid	2.5±0.1	60 ⁰ C	30min	1.54	30.89
20.	Sulfuric acid	2.5±0.1	60 ⁰ C	45min	1.24	24.97
21.	Sulfuric acid	2.5±0.1	60 ⁰ C	60min	1.23	24.73
22.	Sulfuric acid	2.5±0.1	80 ⁰ C	30min	1.69	33.97
23.	Sulfuric acid	2.5±0.1	80 ⁰ C	45min	1.75	35.13
24.	Sulfuric acid	2.5±0.1	80 ⁰ C	60min	1.68	33.79
25.	Sulfuric acid	2.5±0.1	100 ⁰ C	30min	1.82	36.43
26.	Sulfuric acid	2.5±0.1	100 ⁰ C	45min	1.75	35.17
27.	Sulfuric acid	2.5±0.1	100 ⁰ C	60min	1.89	37.93

5.2. Characterization of Pectin

Equivalent weight is used for calculating the anhydrouronic acid content and degree of esterification (DE). It was determined by titration with sodium hydroxide to pH 7.5 using phenolphthalein indicator. The equivalent Wight in mg/ml of pectin extract from passion fruit rind with citric acid was obtained between 135.53-159.23 and with sulfuric acid 140.25-174.82 was obtained.

The methoxyl content or degree of esterification is an important factor in controlling the setting time of pectins, the sensitivity to polyvalent cations, and their usefulness in the preparation of low solid gels, fibres and film. It was determined by saponification of the pectin and titration of the liberated carboxyl groups. The % Methoxyl Content of pectin from passion fruit rind with citric acid was obtained between 12.63%-15.87% and with sulfuric acid 10.89%-12.52% was obtained.

Estimation of anhydrouronic acid content is essential to determine the purity and degree of esterification, and to evaluate the physical properties. Pectin, which is a partly esterified polygalacturonide, contains 10% or more of organic material composed of arabinose, galactose and perhaps sugars. The equivalent weight and methoxyl content value of titration was used. The total Anhydrouronic Acid Content (AUA) of pectin from passion fruit rind with citric acid was obtained between 162.85-200.64 and with sulfuric acid 148.89-171.86 was obtained.

The Degree of Esterification (DE) Content of pectin from passion fruit rind with citric acid was obtained between 31.56% -44% and with sulfuric acid 29.56% -41.39% was obtained.

The IR spectrum (Appendix 6), of the compound displayed absorption band at 2994 cm^{-1} demonstrated the presence of methyl CH_2 stretch of pectin. Absorption band at 1743 cm^{-1} indicated the presence of $\text{C}=\text{O}$ stretch. The absorption bands between 1375 cm^{-1} demonstrated the presence of $\text{C}-\text{O}-\text{O}$ stretch of ester functional group. Absorption band at 1030 cm^{-1} indicated the presence of $\text{C}=\text{O}$ or $\text{C}=\text{C}$ double bond of pectin. Absorption band at 725 cm^{-1} indicate the presence of $\text{C}-\text{H}$ by comparison with literature reports.

5.3. Characterization of F3 compound

The compound PW3 was obtained as yellow solid, soluble in chloroform, show a characteristic colour change to light orange on TLC plate after visualization with I₂ vapor.

Characterization of F3 compound was determined by spectroscopic techniques.

The UV spectrum (Appendix 1) displayed absorption band at λ_{max} (in MeOH) 277nm which showed the π - π^* conjugation.

The IR spectrum (Appendix 5), of the compound displayed absorption band at 2964cm⁻¹, 2927 cm⁻¹ and 2858cm⁻¹ demonstrated the presence of alkane CH₃, CH₃ and CH₂ stretch, respectively. Absorption band at 1737cm⁻¹ indicated the presence of C=O stretch. The absorption bands between 1300-1000, i.e. 1097cm⁻¹ and 1021cm⁻¹ demonstrated the presence of C-C-O stretch of ester functional group. Absorption band at 1263cm⁻¹ indicated the presence of C-O stretch of alcohol. Absorption band at 802 cm⁻¹ indicate the presence of C=C aromatic stretch carbon.

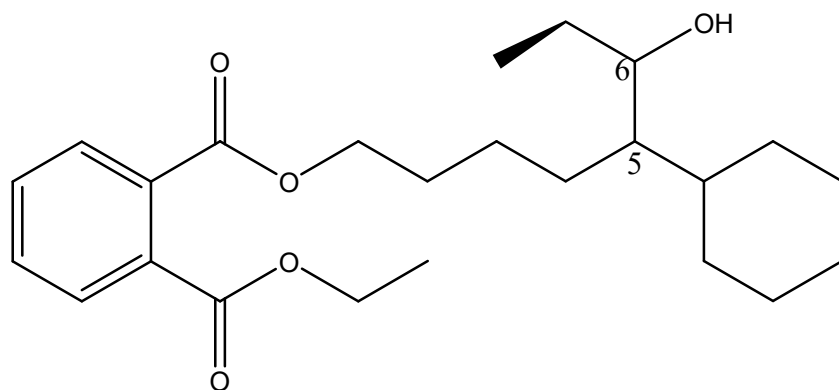
The ¹H NMR spectrum (Appendix 2, Table 3) showed signals at δ 0.90, and δ 1.31 integrating for two methyl protons. An aliphatic methylene group peaks appeared at δ 0.94, 0.96, 1.27, 1.43 and 1.72 each integrating two proton. Other methylene signals appear at δ 4.24 and 4.26 due to oxygenated methylene hydrogen. Peaks at δ 3.69 due to oxygenated methane. One methine Peaks appeared at δ 1.24 in cyclohexane. Signals at δ 1.43, 1.63 and 1.69 correspond to cyclohexane methylene protons. Signals appeared at δ 7.74 and 7.55 indicate the presences of aromatic ring. Peak observed δ 4.1 indicates the OH proton.

The ¹³C NMR and DEPT-135 (Appendix 3) indicated that PE3 has 18 carbon atoms. The spectra showed two aliphatic methyl carbons at δ 10.97 and 14.07, four aliphatic methylene carbons at δ 23.00, 28.93, 29.72 and 30.36, three cyclohexane methylene at δ 23.74, 27.21 and 34.03, two oxygenated methylene carbon atom at δ 62.11 and 68.61, one aliphatic methine at δ 38.72 and one cyclohexane methine at δ 31.63, two methine at δ 128.81 and 130.90, one oxygenated methine at δ 68.88, and two quaternary carbon at δ 132.45 and 167.79.

Table 3: ^{13}C NMR, DEPT-135 and ^1H NMR spectral data of Compound F3

Position	^{13}C δ (ppm)	DEPT δ (ppm)	^1H δ (ppm)	Remark
1.	132.45	-	-	Quaternary
2.	128.81	128.8	7.7	CH
3.	130.90	130.9	7.5	CH
4.	167.79	-	-	Quaternary
5.	68.88	68.9	3.4	CH
6.	68.16	68.2	4.3	CH_2
7.	62.11	62.1	4.2	CH_2
8.	38.72	38.7	1.0	CH
9	31.63	36.6	1.2	CH
10	34.03	34.0	1.6	CH_2
11	30.36	30.4	1.7	CH_2
12	29.72	29.7	1.3	CH_2
13	28.93	28.9	1.4	CH_2
14	27.21	27.2	1.6	CH_2
15	23.74	23.7	0.9	CH_2
16.	23.00	23.0	1.4	CH_2
17.	14.07	14.1	1.3	CH_3
18.	10.97	10.9	0.9	CH_3

Based on the above evidence the following structure proposed for PE3.



5-cyclohexyl-6-hydroxyoctyl ethyl phthalate

Chapter 6

5.0. Conclusion and Recommendation

Passion fruit rind is a high source of lower-ester pectin and natural product. In this study pectin extracted using citric acid and sulfuric acid in different pH, temperature and time. The best extraction method of pectin was citric acid and it have high % yield than sulfuric acid extract by any condition used. The characterizations of pectin data indicate that pectin extract from passion fruit rind was low-ester pectin, with DE lower than 50%. In this study passion fruit rind chloroform extract gave 5-cyclohexyl-6-hydroxyoctyl ethyl phthalate.

Until now no report were found in Ethiopia, either on pectin or other natural products extraction from Passion fruit rinds. This shows that, this plant is not known in terms of its uses. Thus further investigation should be carried out to benefit from its uses or for academic intention as well. Since this fruit has pectin and natural product as demonstrated by this research, it is possible to isolate many compounds from this plant. Especially commercial pectin can be manufactured from the plant rind in Ethiopia.

Chapter 7

6.0. Reference

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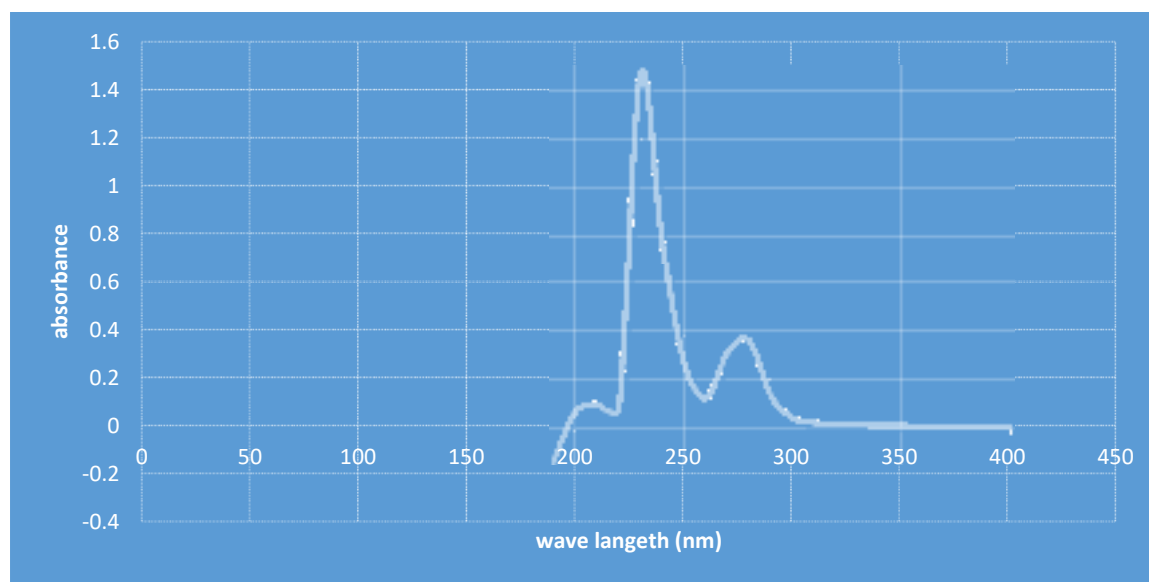
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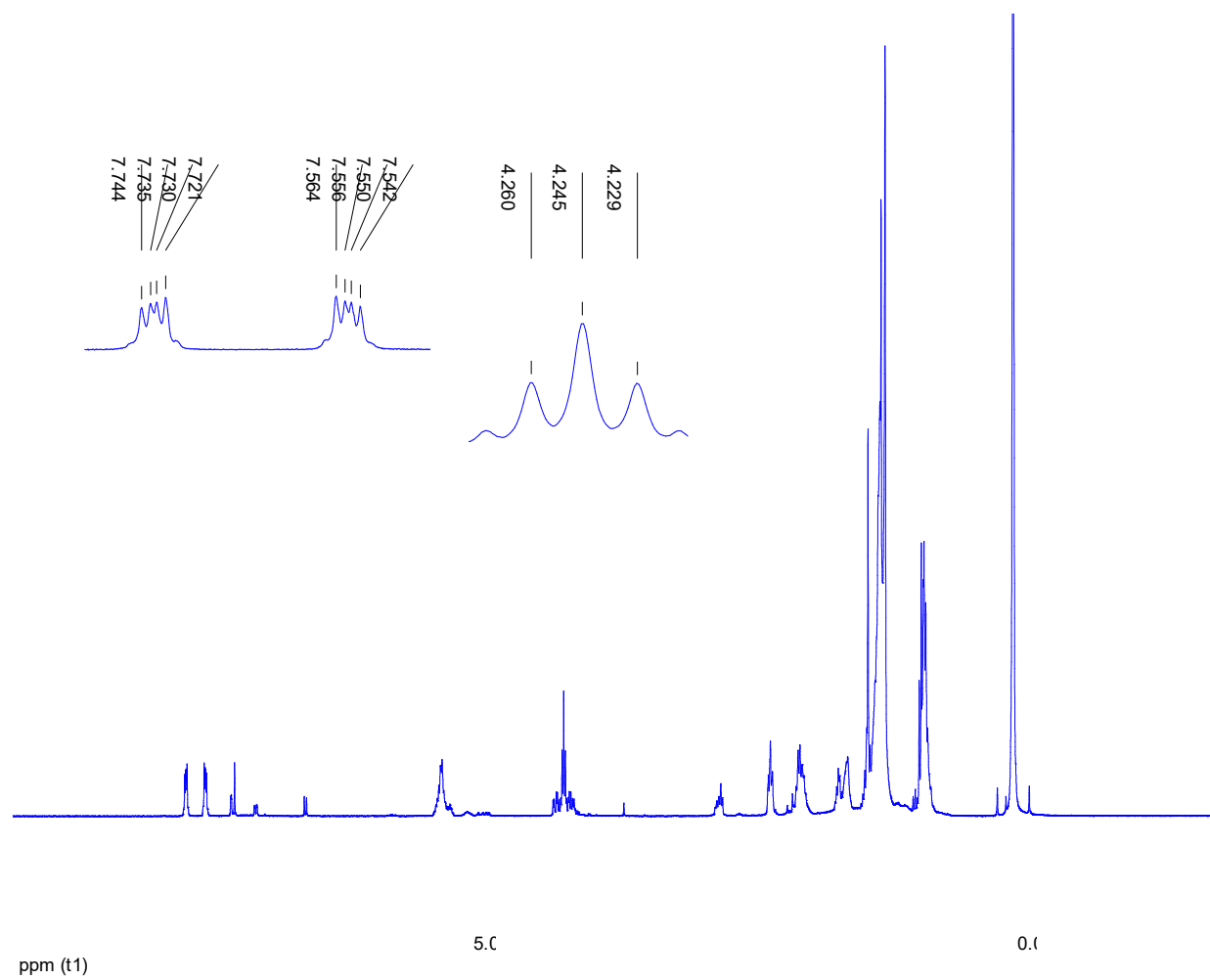
Chapter 8

7.0. Appendices

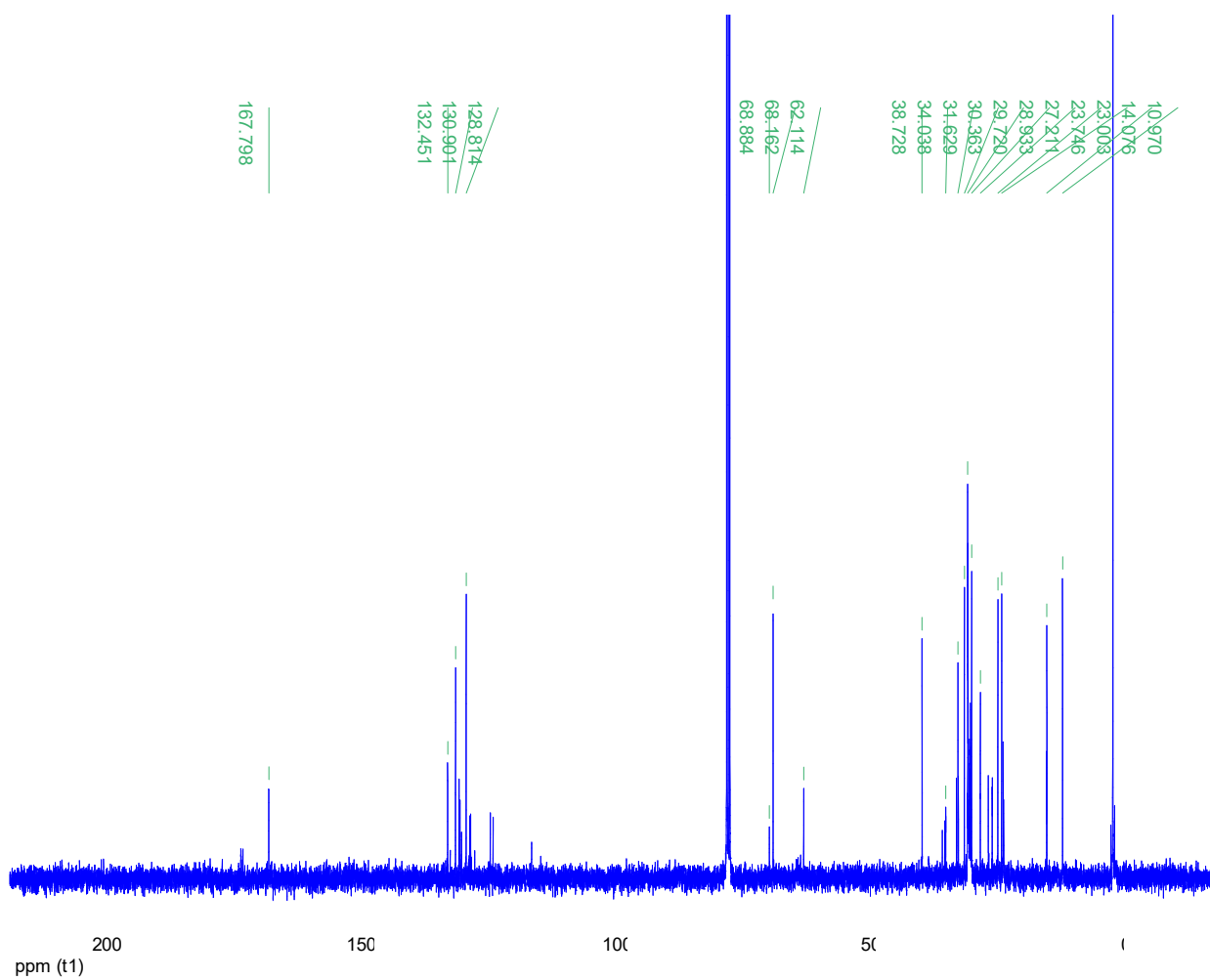
Appendix 1. UV-VIS spectrum of compound PE3



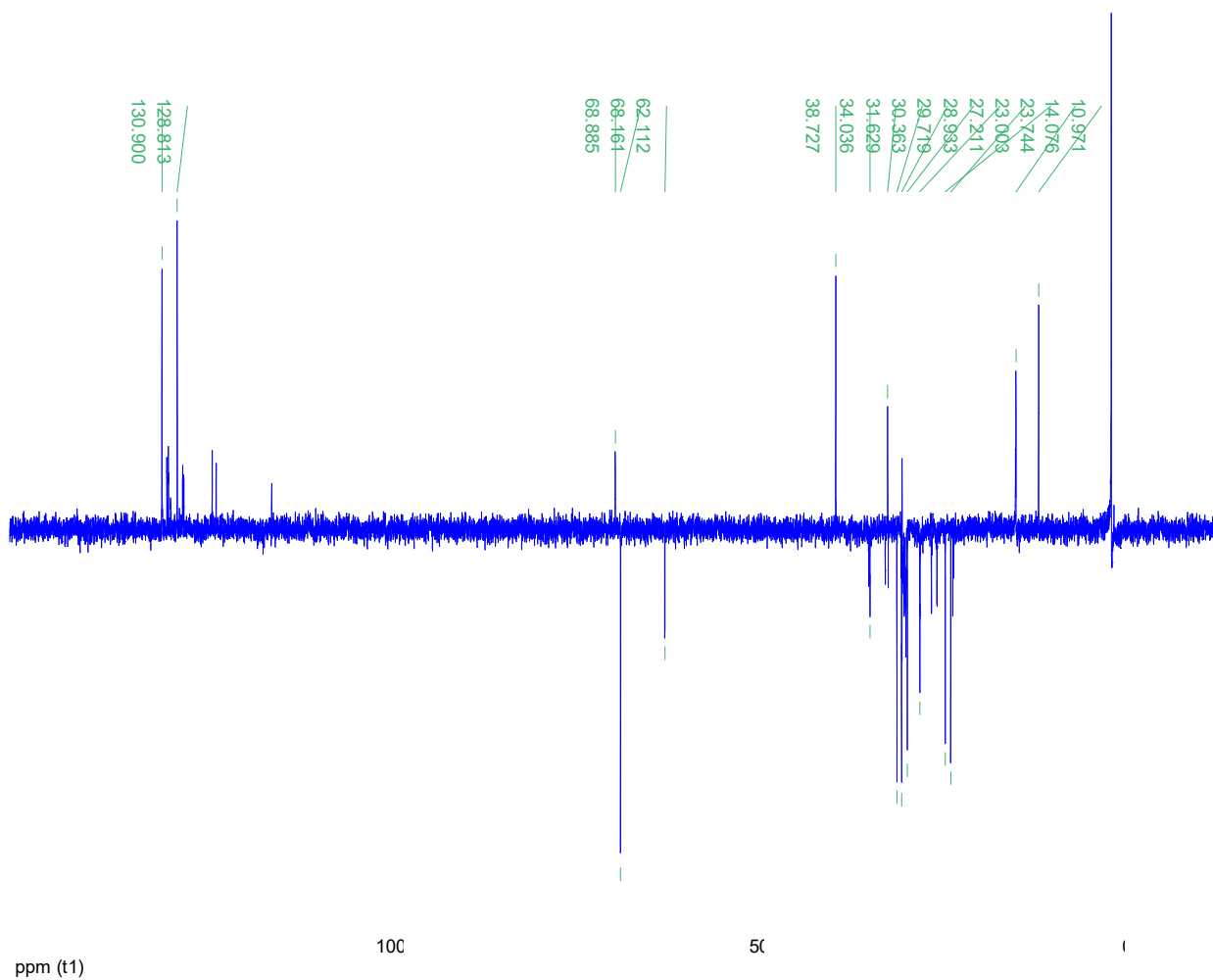
Appendix 2: ^1H NMR spectrum of compound PE3 (CDCl_3)



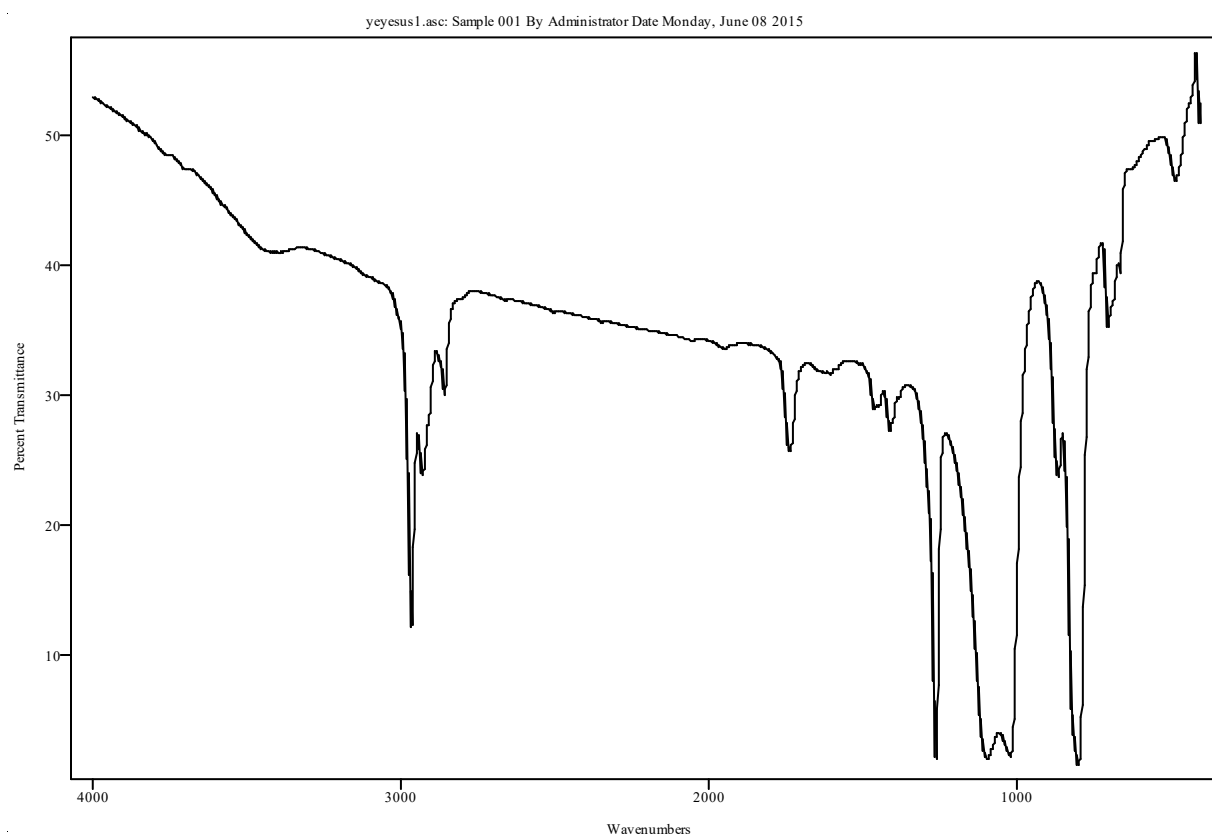
Appendix 3: ^{13}C NMR spectrum of compound PE3 (CDCl_3)



Appendix 4: DEPT-135 NMR spectrum compound PE3 (CDCl₃)



Appendix 5: IR spectrum of compound PE3



Appendix 6: IR spectrum of pectin

