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Al-Quds University

Microemulsion containing natural arbutin nanoparticles

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Thesis Approval

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Jerusalem – Palestine

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Declaration

I certify that this thesis submitted for the degree of master is my own research, expect where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for higher degree to any other university or institution.

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Abstract

Fruit peels are produced in enormous quantities as a byproduct of pear product to produce Arbutin, along with chlorogenic acid and rutin, it is one of the phenolic chemicals found in pear fruits and peels. In this research, we focused on pear peels since the pear peel is a bio-waste and a byproduct and we could achieve many objectives. Firstly, the pear peel is extracted by sonication method using ethanol 70% as a solvent, in which 50% of the extract was obtained. Secondly, The amount of arbutin in the natural extract has appeared in a high quantity according High-performance liquid chromatography (HPLC) analysis. Thirdly, the anti-glycation production of extract was determined to be 18% at 350 ppm of the extract by using an in vitro glucose-bovine serum albumin (BSA) test. Then the antioxidant effects were evaluated and found to be between 32% to 39.7% using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method. A Folin-Ciocalteau test was used to determine total phenolic content (TPC) which was 64.4 mg/GAE , and a colorimetric assay was used to determine total flavonoid content (TFC)that was 13.9099 mg/QE. Finally, the pear extract was loaded on micro-emulsion, which is a thermodynamic stable preparation in order to increase the bioavailability and permeability through skin. Pseudo-ternary phase diagrams were prepared by titration method and plotted to identify the micro-emulsification regions. The micro-emulsion consisted of Isopropyl myristate (IPM) as oil phase, Polysorbate20 (Tween20) as surfactant, ethanol as Co-surfactant phase and the pear peel extract as water phase. Three regions appeared in the pesdo-ternary phase diagram. Those were the one phase micro-emulsion region, the two-phase emulsion region and the three-phase region. Micro-emulsion region occupied the largest area of the phase diagram.

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Abbreviations

AGEs: Anti-glycation end products

TFC: Total Flavonoids Content

TPC: Total phenolic Content

DPPH:1,1-diphenyl 2-picrylhydrazyl (DPPH•) radical

BSA: bovine serum albumin

GAE: Gallic Acid Equivalent

QE: Quareciten

HPLC: High Performance Liquid chromatography

O/W: Oil in water

W/O: Water in oil

IPM: Isopropylmyristate

CHAPTER ONE

INTRODUCTION

Chapter One:

Introduction

1.1 The importance of Pears (pyrus spp)

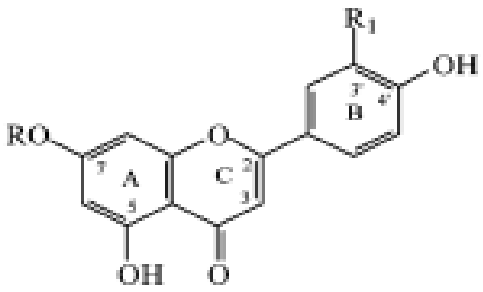
A sweet, juicy fruit with a buttery, glittery texture. It has an elongated basal portion, a diameter of 1–4 cm, and a height of 10–17 m. It has 2–12 cm long, glossy green, alternately placed leaves with a broad oval shape. The pulp is fragrant, white, sweet, and contains a few small brown seeds. It is available fresh, canned, dried, and juiced). As shown in figure (1.1) the fruit is a pyriform pome with a deciduous or persistent calyx, 4–12 centimeters long, dry, gritty, and greenish in color (Ruaa et al 2020)

Pear (Pyrus spp.) is found all over the world, but especially in Europe and Asia. Asian pears are primarily grown in Eastern Asia, specifically in Korea, China, and Japan. Fresh fruit and processed foods such as juice, jellies, and jams are commonly consumed. Fruit peels are produced in massive quantities as a byproduct of pear product manufacturing process. A method is developed for recovering lipids from pears' fruit peels recently, including polyunsaturated fatty acids, tocopherols, and sterols. Arbutin, along with chlorogenic acid and rutin, is a phenolic chemical found in pear fruits (Ramadan et al2003)

Figure (1.1) Photo of pyrus (spp) plant (Ruaa et al 2022)

1.2 Chemical and Phytochemical Screening of pear

The chemical composition of pears varies depending on their location. Arbutin, oleanolic acid, ursolic acid, chlorogenic acid, epicatechin, and rutin were found to be the most abundant monomeric compounds in various pear cultivars' peel and flesh. Figure 1.2 summarizes the most important functional pear compounds. Pears are primarily composed of water (approximately 80%), sugar and fructose (15%), and fiber (approximately 2%): Arbutin, chlorogenic acid, p-coumaroylquinic acid, p-coumaroylmalic acid, dicaffeoylquinic acids, vanillic acid derivatives, catechin, epicatechin, proanthocyanidins, cyanidin 3-O-galactoside, the 3-O-glycosides of quercetin, isorhamnetin, and kaempferol, and some nonglycosylated flavones and flavonols) these compounds according to Previous studies were foundin pear skins, pear flowers and other parts of pear trees (Figure 1.2). The phenolic content of pear skins is much higher and more diverse than that of the fruit flesh. Such polyphenols in pear materials have also been reported in studies using liquid chromatography (LC) or liquid chromatography with mass spectrometric detection (LC-MS), and the chemical composition of pears is varied including Arbutin, oleanolic acid, ursolic acid, chlorogenic acid, epicatechin, and rutin were found to be the most abundant monomeric compounds in various pear cultivars' peel and flesh(Rychlinska et al 2012),(Koleckar,2008).



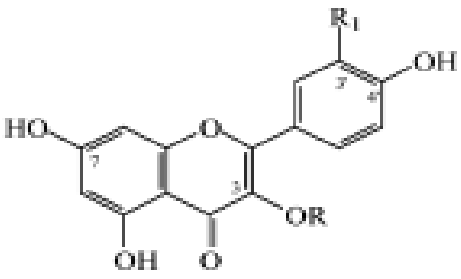
Flavones and their glycosides

Apigenin: R=R_1=H, Mr 270

Luteolin: R_1=OH, R=H, Mr 286

Chryseoriol: R_1=OMe, R=H, Mr 300

Their 7-O-glycosides: R=glycosyl



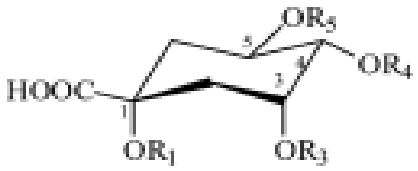
Flavonols and their glycosides

Kaempferol: R_1=H, Mr 286

Quercetin: R_1=OH, Mr 302

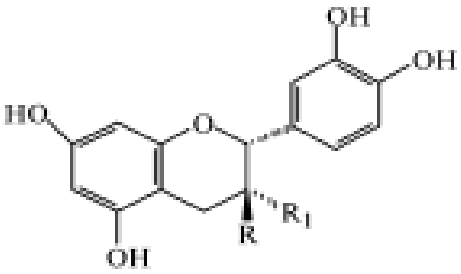
Isorhamnetin: R_1=OMe, Mr 316

Their 3-O-glycosides: R=glycosyl



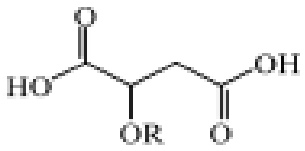
Quinic acid, R_1=R_3=R_4=R_5=H, Mr 192

Caffeoylquinic acids: all the Rn=caffeoyl as the name indicated, and the remained Rn=H

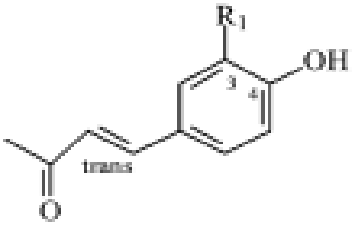


Catechin: R=OH, R_1=H, Mr 290

Epicatechin: R=H, R_1=OH, Mr 290



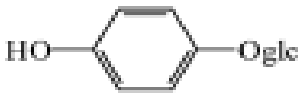
Malic acid, R=H, Mr 134



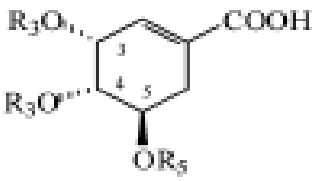
Trans-hydroxycinnamoyl

p-Coumaroyl: R_1=H

Caffeoyl: R_1=OH



Arbutin, Mr 272



Shikimic acid, R_3=R_4=R_5=H, Mr 174

Figure (1.2):structures of the phenolic compounds in the pyrus

(Rychlinska.et al 2012)

1.2.1 Arbutin:

Arbutin (4-hydroxyphenyl glucopyranoside) is a phenolic compound found in pear peel that is used as a whitening agent in cosmetics (Cho, J.-Yet et al 2011).Arbutin's high hydrophilicity reduces its permeability across the stratum corneum, which improves skin penetration and the efficacy of a hyperpigmentation agent (Migas,P et al 2015). Cell penetrating peptide conjugated liposomes were also used to improve transdermal delivery of Polygonum aviculare L. extract ، Arbutin (4-hydroxyphenyl glucopyranoside) is widely used in cosmetic products as a whitening agent due to its ability to inhibit tyrosinase. There are two anomeric forms of arbutin: α- and β - and -arbutins (Fig. 1.3) (fig. 1.4).Both have been shown ability to inhibit tyrosinase activity in mushrooms. In ethanol extracts, (Cho, J.-Yet et al 2011).Arbutin is relatively stable. And in cosmetics in the 4-8 pH range (Migas,P et al 2015), but it may undergo partial hydrolysis in aqueous extracts (Migas,P et al 2015).

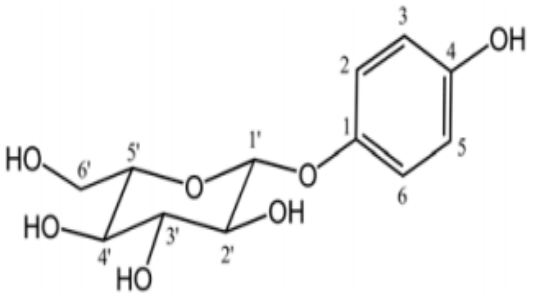


Figure (1.3): chemical structure of Arbutin (Cho, J.-Yet et al 2011)

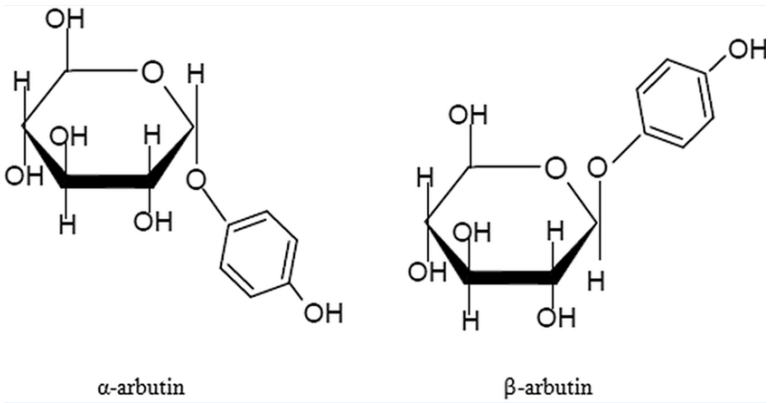


Figure (1.4): isoforms of arbutin's structures (α-arbutin and β-arbutin)(Hazman et al 2020)

1.3 Traditional Uses of pear

Traditional Chinese medicine uses the aerial and floral parts of the pyrus tree because they have anti-inflammatory effect. Pears are low in calories, have antipyretic, diuretic, and antitussive effects; they accelerate digestion and intestinal peristalsis; change blood pressure; and have these effect. Use the bark and leaves of this plant to speed up wound healing.

Because of the astringent qualities of the tree's buds, bark, and leaves, the Arabs utilize them as household tools. Spasmolytic and analgesic Medications. In traditional medicine, pear flowers are used for therapeutic purposes (Ruaa et al 2020).

1.4. Pharmacological activity of Pyrus

Since ancient times, pears have been used as a significant medicinal plant. It contains many antioxidants, which have dozens of health advantages and can fight many degenerative diseases. The pear tree's entire body is rich in nutrients and has numerous health benefits, including hepatoprotective, anti-diabetic, sedative, anti-inflammatory, antioxidant, antipyretic, hypolipidemic, antiaging, hypoglycemic, analgesic, antitussive, spasmolytic, anti-diarrheal, and anti-microbial properties due to the presence of quercetin, copper, and vitamin C, which aid in preventing celluar damage from free radicals. One of the most prevalent pear diseases, fire blight, is brought on by the bacterial pathogen Erwinia amylovora (Ruaa et al 2020).

1.4.1 Antioxidant activity of pyrus

A major part of the dermal extracellular matrix (ECM), which provides human skin its tensile strength, is the fibrous protein collagen (Imeh et al 2002). Procollagen serves as the precursor for collagen, which is then generated by ECM fibroblasts(Chen et al 2006).

Proline and lysine residues in procollagen are hydroxylated, which causes the protein to be released into the extracellular matrix and polymerized into collagen fibers.

The callus extract from Pyrus pyrifolia var. culta markedly boosted fibroblast proliferation,which encourages collagen formation (Chen et al 2007).

It has also been noted that Pyrus pyrifolia var. culta extracts reduce glycation.

Glycation of proteins is brought on by the buildup of non enzymatic byproducts from protein reactions with glucose and other reducing sugars (Cui et al 2005).

1.4.2 Whitening agent properties of *pyrus*

Tyrosinase activity was studied to evaluate the impact of the Pyrus pyrifolia var. culta extract on melanogenesis; substances with whitening effects are often anti-oxidant active (Robards et al 1999).The multifunctional copper-containing enzyme tyrosinase catalyzes the conversion of L-tyrosine to L-DOPA and the oxidation of L-DOPA to dopaquinone, two crucial steps in the manufacture of melanin (Imeh et al 2002), (Escarpa et al 2001). The extract strongly inhibited DOPA auto-oxidation in melanogenesis but did not decrease tyrosinase activity at the concentrations examined in this study. These results suggest that the antioxidant capacity of the extract may be a major factor in the reduced melanin production.

1.5 Antiglycation and Diabetes complications

Dexamethasone-induced diabetic rats were used to examine the hypolipidemic and hypoglycemic effects of ethanol and ethyl acetate extracts of Pyrus communis fruits. Increased hepatic glucose synthesis and peripheral tissue insulin resistance are blamed for the hyperglycemia brought on by glucocorticoids.

Rats with diabetes have lower body weights than normal rats and high blood sugar levels. Oral administration of Pyrus communis ethanol and ethyl acetate extracts significantly reduced blood glucose levels due to potentiation of the plasma insulin effect by increasing its release from bound insulin or pancreatic insulin secretion from existing Langerhans islet -cells.

The hypoglycemic effect of the extract may be due to an independent mechanism other than insulin secretion, such as inhibiting intestinal glucose absorption or inhibiting endogenous glucose production, or it may be due to increased glucoseutilization by peripheral tissues. The main cause of cardiovascular disease in diabetesis abnormalities in the lipid profile caused by atherosclerosis. (Ruaa et al 2020).

1.6 Microemulsion

Microemulsions are dispersions of two immiscible liquids, such as oil and water, that are fluid, thermodynamically stable, and optically clear .

When a surfactant, or more frequently a combination of surfactants and cosurfactants, decreases the oil/water interfacial tension to extremely low values (typically less than 0.001

dynes/cm), thermal movements can spontaneously separate the two immiscible phases, resulting in microemulsions (Klier et al .,2000).

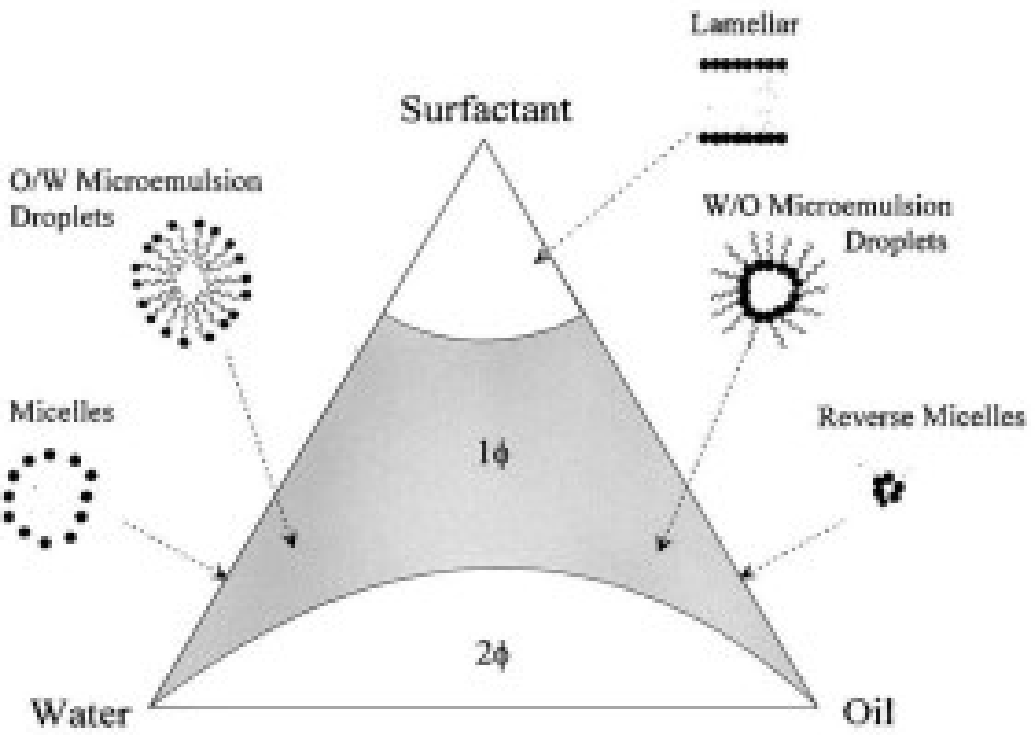


Figure (1.5): Oil/surfactant/water system hypothetical phase diagram for the micro-emulsion and emulsion phases (Lawrence et al 2000).

1.7. Surfactant

A "surface-active agent" is a chemical that exhibits some interfacial or surface activity. Not all amphiphiles display this tendency, it should be noted; only those amphiphiles with nearly equal hydrophilic and lipophilic tendencies are likely to gravitate toward the surface or contact. It will not occur if the amphiphilic molecule is overly hydrophilic or hydrophobic since it will stay in one of the phases (SalaGer et al., 2002).

1.7.1. Classification of Surfactants:

In water, anionic surfactants split into an amphiphilic anion and a cation, which is typically an alkaline metal (Na+, K+) or quaternary ammonium.

The majority of Surfactant are employed in their production. The second most produced industrial substance, nonionic surfactants, accounts for around 45% of total output. Because they contain a nondissociable hydrophilic group, such as alcohol, phenol, ether, ester, or amide, in an aqueous solution, they do not ionize.

These nonionic surfactants contain a considerable number of polyethylene glycolchains, which are produced when ethylene oxide is polycondensed, and these chains make the nonionic surfactants hydrophilic. As an amphiphilic cation and an anion, typically of the halogen type, cationic surfactants dissociate in water. Molecules that are either amphoteric or zwitterionic show both.

1.8. Phase behavior

A mixture's composition and phase behavior can be limited using phase diagrams.
Ternary phase diagrams are helpful for examining the phase behavior of fundamental microemulsion systems composed of oil, a surfactant, and a cosurfactant.
An entire unit of the meticulous component is shown in each corner of the diagram the majority of the time, though, a cosurfactant or drug is an additional element in micro-emulsions.
When there are four or more components, pseudoternary diagrams are made, and each corner represents a binary combination of two components, such as a surfactant and Co-surfactant, water/drug, or oil/drug (Ritika et al.,2012).

1.9 Problem statement:

Phenolic compounds especiallyβ-arbutins in pear peel,require an effective method to be extracted in high quantity with much lesseffort. Due to the low permeability of β-arbutins, we need to develop a β-arbutins delivery to reach across the stratum corneum most common and dangerous diseases. Researchers are continuously working to create natural anti-glycation drugs that are of low-cost and low-risk. This agriculture by-product) along with studying of total phenol, antioxidant, total flavonoids, and antibacterial effects.

1.10 Objectives of this study

The main goals of this research are:

1- studying in-vitro anitglycation assay, antioxidant activity, total phenolic and total flavonoids content of pear peel extract.
2- HPLC analysis of Arbutin in pear peel extract.
3- Extract of pear peel by sonication method.
4- Construction of ternary phase diagram.

CHAPTER TWO

LITERATURE REVIEW

Chapter Two:

Literature Review:

The main phenolic substances in pears include chlorogenic acid, arbutin, and epigallocatechin gallate. They are phenolic chemicals, which act as antioxidants. Chlorogenic acid, the most potent antioxidant found in pears, may potentially function as a potential chemo preventive agent, supporting the prevention of cancer and cardiovascular disease as well as other chronic diseases, boosting the immune system and reducing the negative effects of chemotherapy drugs. Arbutin, another important phenolic compound in pears, works as an antibiotic to stop the spread of fire blight.

The peel typically contained more phenolic chemicals than the flesh (table 2.1) (Öztürk et al 2015).

Hydrate were found to be minor phenolic compounds in the peel and flesh, While arbutin and chlorogenic acid were important phenolic compounds found there (table 2.1). (table 2.2). In the meat, catechin levels varied from 40.0 to 543.8 mg kg^{-1}, and in the peel, they were 42.4 to 695.2 mg kg^{-1}.The flesh's epicatechin concentration ranged from 11.47 to 243.1 mg kg^{-1}, whereas the peel's level ranged from 12.6 to 315.4 mg kg^{-1} (table 2.2). (Öztürk et al 2015).

The fruit of Pyrus communis, also known as the pear, has a wide range of pharmacological characteristics. The wide spectrum of biological actions of natural bioactive chemicals, including phenols, tannin, and flavonoids, which are key secondary metabolites in plants, is validated by scientific research on these metabolites from pears. Different solvents, including chloroform, ethyl acetate, ethanol, and water, are employed during the extraction process to maximize the concentration of these agents in the extract. The goal of the current study was to measure the contents of tannin, flavonoids, and total phenolics. Observations show that ethanol and ethyl acetate extracts have very high (Velmurugan C et al 2014) as shown in table (2.4)

Table 2.1: Content of hydroxycinnamic acid derivatives (chlorogenic, caffeic, and p-coumaric acid) in peel and flesh of pear cultivars (mg kg^{-1})(Öztürk et al 2015)

	Chlorogenic acid[x]		*Caffeic acid*[x]		p-*coumaric acid*[x]	
Cultivars	*Flesh*	*Peel*	*Flesh*	*Peel*	*Flesh*	*Peel*
Istanbul	164.8 ± 8.1	320.7 ± 17.9	4.5 ± 0.9	8.8 ± 1.2	3.8 ± 0.7	3.9 ± 0.4
Seker	223.1 ± 9.8	342.4 ± 18.1	24.9 ± 1.1	29.5 ± 2.9	3.7 ± 0.6	3.9 ± 0.2
Kıs	46.0 ± 5.8	69.3 ± 7.5	12.1 ± 1.1	12.7 ± 1.2	3.9 ± 0.5	4.2 ± 0.8
Bardak	47.6 ± 4.9	69.4 ± 7.6	12.1 ± 1.2	12.7 ± 1.2	3.4 ± 0.4	3.9 ± 0.3
Fırıncık	164.9 ± 5.4	225.9 ± 13.9	15.3 ± 2.1	18.7 ± 1.8	1.9 ± 0.5	1.9 ± 0.5
Esek	61.6 ± 5.2	78.2 ± 8.5	77.7 ± 3.5	88.5 ± 8.8	0.3 ± 0.1	0.3 ± 0.2
Pazar	164.8 ± 9.8	375.7 ± 11.9	176.8 ± 9.8	238.4 ± 12.6	0.2 ± 0.2	0.2 ± 0.4
Yaz Ziraati	320.9 ± 8.8	344.9 ± 15.9	12.5 ± 1.3	17.3 ± 1.2	2.2 ± 0.3	2.5 ± 0.3
Karpuz	46.0 ± 6.2	76.4 ± 9.7	12.2 ± 1.2	14.2 ± 1.3	2.4 ± 0.4	2.2 ± 0.4
Sarıkum	891.9 ± 31.2	1348.4 ± 32.8	54.6 ± 3.2	128.7 ± 9.6	0.3 ± 0.1	0.4 ± 0.1
Kara	15.8 ± 3.1	67.6 ± 8.5	180.7 ± 9.9	199.3 ± 8.9	1.9 ± 0.1	2.7 ± 0.1
Rıza	17.8 ± 3.8	58.7 ± 5.2	258.3 ± 10.1	317.6 ± 19.2	0.8 ± 0.1	0.9 ± 0.1
Dalkıran	100.6 ± 7.5	300.5 ± 9.8	72.1 ± 2.6	103.5 ± 9.6	0.4 ± 0.2	0.5 ± 0.1
Deveci*	47.5 ± 6.1	36.8 ± 3.2	10.3 ± 1.1	18.9 ± 1.9	1.7 ± 0.3	1.8 ± 0.3
Williams*	18.0 ± 2.9	21.0 ± 2.9	9.1 ± 0.9	10.3 ± 0.8	1.3 ± 0.1	1.3 ± 0.4
Abbe Fetel*	424.6 ± 28.2	457.7 ± 21.2	4.9 ± 0.5	14.4 ± 1.8	0.3 ± 0.1	0.4 ± 0.1
Santa Maria*	242.6 ± 19.1	459.8 ± 25.4	16.5 ± 1.2	15.8 ± 1.3	0.4 ± 0.1	0.4 ± 0.1
Mean	176.4 ± 9.8	273.7 ± 12.9	56.2 ± 3.0	73.5 ± 5.0	1.7 ± 0.3	1.8 ± 0.3
C.V (%)	5.55	4.71	5.33	6.82	17.6	16.7

*: Standard pear cultivars;
[x]: $n = 6$ (3 replications × 2 different measurements for each replicate).

Table 2.2: Arbutin and flavan-3-ol concentrations (mg kg^{-1}) in the peel and flesh of several cultivars of pears (Öztürk et al 2015)

	Arbutin[x]		*Catechin*[x]		*Epicatechin*[x]	
Cultivars	*Flesh*	*Peel*	*Flesh*	*Peel*	*Flesh*	*Peel*
Istanbul	12803.1 ± 27.9	17422.9 ± 83.1	248.1 ± 12.1	306.2 ± 12.3	140.6 ± 5.2	145.9 ± 4.9
Seker	13153.7 ± 31.9	14637.4 ± 77.2	462.8 ± 23.6	502.0 ± 23.6	86.5 ± 2.9	97.5 ± 3.2
Kıs	10952.6 ± 34.3	14919.2 ± 89.8	115.9 ± 7.6	123.5 ± 6.5	98.8 ± 2.8	116.2 ± 3.1
Bardak	6023.2 ± 28.2	13853.4 ± 77.6	88.9 ± 4.6	102.3 ± 2.3	35.1 ± 1.2	32.4 ± 0.9
Fırıncık	13270.8 ± 22.7	15565.8 ± 83.2	174.4 ± 6.1	186.3 ± 6.5	69.4 ± 2.3	78.0 ± 2.1
Esek	9431.9 ± 25.3	14875.4 ± 61.4	139.4 ± 6.5	128.1 ± 5.6	57.5 ± 2.5	61.3 ± 2.6
Pazar	6182.6 ± 22.4	15175.3 ± 77.6	543.8 ± 25.3	695.2 ± 26.5	41.5 ± 3.1	43.8 ± 1.9
Yaz Ziraati	8464.7 ± 29.8	15199.8 ± 81.0	39.3 ± 3.1	42.4 ± 2.1	69.1 ± 5.4	86.8 ± 2.0
Karpuz	11036.1 ± 35.3	15565.9 ± 72.5	157.5 ± 9.6	166.2 ± 4.9	78.9 ± 5.2	86.0 ± 3.1
Sarıkum	3779.1 ± 26.7	67446.3 ± 93.7	56.3 ± 2.6	75.2 ± 3.8	114.7 ± 5.9	315.4 ± 15.9
Kara	5669.5 ± 21.2	22403.1 ± 51.6	63.2 ± 2.8	85.0 ± 6.5	119.6 ± 8.7	261.2 ± 6.5
Rıza	11602.8 ± 39.4	10433.0 ± 89.6	40.0 ± 2.1	265.8 ± 11.2	11.4 ± 0.1	12.6 ± 0.2
Dalkıran	14175.7 ± 37.5	14171.8 ± 86.4	174.1 ± 12.1	147.9 ± 14.2	174.7 ± 6.2	191.1 ± 3.5
Deveci*	15412.0 ± 45.9	13049.6 ± 87.5	55.9 ± 2.5	61.7 ± 6.1	243.1 ± 8.1	227.2 ± 7.9
Williams*	3195.0 ± 20.2	4890.0 ± 59.5	45.4 ± 1.2	48.5 ± 3.5	47.8 ± 3.6	59.4 ± 3.6
Abbe Fetel*	3067.7 ± 19.1	16031.2 ± 38.6	107.3 ± 6.4	117.0 ± 8.7	15.5 ± 0.5	19.6 ± 0.8
Santa Maria*	15209.9 ± 44.1	13270.0 ± 48.4	364.9 ± 10.1	374.8 ± 12.7	108.5 ± 4.2	118.1 ± 4.1
Mean	9613.5 ± 30.1	17582.9 ± 74.0	166.3 ± 8.1	201.7 ± 9.2	88.9 ± 4.0	114.8 ± 3.9
C.V (%)	0.31	0.42	4.87	4.56	4.49	3.39

*: Standard pear cultivars;
[x]: $n = 6$ (3 replications × 2 different measurements for each replicate).

Table 2.3: Rutinhydrate and rutin-tri-hydrate flavonol glycoside content (mg kg^{-1}) in pear cultivars' peel and flesh (Öztürk et al 2015).

	Rutinhydrate[x]		*Rutin-tri-hydrate*[x]	
Cultivars	*Flesh*	*Peel*	*Flesh*	*Peel*
Istanbul	0.29 ± 0.1	0.30 ± 0.1	0.004 ± 0.01	0.742 ± 0.04
Seker	0.28 ± 0.1	0.29 ± 0.1	0.053 ± 0.01	0.054 ± 0.02
Kıs	0.40 ± 0.1	0.59 ± 0.2	0.005 ± 0.01	0.323 ± 0.04
Bardak	1.19 ± 0.2	1.27 ± 0.2	0.418 ± 0.02	0.848 ± 0.04
Fırıncık	0.28 ± 0.1	0.29 ± 0.1	0.006 ± 0.01	0.006 ± 0.02
Esek	0.66 ± 0.3	0.78 ± 0.2	0.022 ± 0.01	0.022 ± 0.01
Pazar	0.43 ± 0.2	0.50 ± 0.1	0.005 ± 0.01	0.110 ± 0.01
Yaz Ziraati	0.40 ± 0.1	0.47 ± 0.2	0.004 ± 0.01	0.365 ± 0.02
Karpuz	0.34 ± 0.2	0.39 ± 0.1	0.324 ± 0.02	0.364 ± 0.02
Sarıkum	1.31 ± 0.2	1.38 ± 0.2	0.046 ± 0.03	0.055 ± 0.01
Kara	0.53 ± 0.1	0.59 ± 0.1	0.073 ± 0.01	0.075 ± 0.02
Rıza	0.89 ± 0.2	0.98 ± 0.2	0.075 ± 0.01	0.082 ± 0.03
Dalkıran	0.75 ± 0.1	0.82 ± 0.1	0.036 ± 0.01	0.041 ± 0.01
Deveci*	0.88 ± 0.1	1.00 ± 0.2	0.169 ± 0.03	0.181 ± 0.01
Williams*	0.14 ± 0.1	0.15 ± 0.1	0.061 ± 0.02	0.056 ± 0.02
Abbe Fetel*	0.03 ± 0.1	0.04 ± 0.1	0.007 ± 0.01	0.006 ± 0.01
Santa Maria*	1.34 ± 0.2	1.48 ± 0.2	0.087 ± 0.01	0.080 ± 0.03
Mean	0.59 ± 0.14	0.66 ± 0.16	0.13 ± 0.014	0.20 ± 0.021
C.V (%)	24.92	24.95	10.76	10.5

*: Standard pear cultivars;
x: $n = 6$ (3 replications × 2 different measurements for each replicate).

Table 2.4: Total phenol ad flavonoid content of different extracts of fruits of Pyrus communis L. (Velmurugan C et al 2014)

Extracts	**Total phenol mg GA/g of extract**	**Total flavonoid mg Ru/g of extract**
Chloroform	8.38±0.17[d]	9.72±0.59[h]
Ethyl acetate	49.33±0.08[a]	54.77±0.41[e]
Ethanol	46.63±0.12[b]	52.92±0.94[e]
Aqueous	15.27±0.03[c]	16.92±0.38[g]

The oxidation processes that are influenced by reactive oxygen species or ambient oxygen can be slowed down or stopped by antioxidants. They are used to stabilize polymeric materials as well as petrochemicals, food, cosmetics, and pharmaceuticals. The body's defense mechanism against diseases brought on by the attack of free radicals includes antioxidants (Pioschi et al.,2011).

The antioxidative system includes both enzymatic and non-enzymatic mechanisms. Among the non-enzymatic parts of the body are cartenes, tocopherol, and ascorbic acid (vitamin C). Superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase

(APX), glutathione reductase (GR), and polyphenol oxidase (PPO), among other enzymes, are among the enzymatic components. This antioxidant system's function is to eliminate the damaging radicals produced during oxidative stress, enabling the plants to withstand these conditions(Kumar et al.,2014).

The 2-Diphenyl-1-picrylhydrazyl (DPPH) approach relies on the stabilizing free radical DPPH being reduced. Maximum absorption from the free radical DPPH with an odd electron occurs at 517 nm (purple colour). When antioxidants interact with DPPH, a stable free radical, it pairs off with a hydrogen donor (such as an antioxidant that scavenges free radicals) and is reduced to the DPPHH, which lowers the absorbance of the DPPH. Radical to the DPPH-H form causes decolorization (yellow color), depending on how many electrons are trapped. (Shekhar et al.,2014).

"80% ethanol and an acidic atmosphere are used in a simple process to separate and purify arbutin from pears." As a result, very pure arbutin is produced (pH 3.0). The bulk of the arbutin (78%) was found in the fraction of water and ethyl acetate. Arbutin was further purified by preparative HPLC and Diaion HP-20column chromatography after being removed from the water layer. The recovered 60% of the isolated arbutin had a purity of more than 99% (Cho, J.-Y et al 2011).

Arbutin was chosen for the micellar delivery trials because of its superior performance in lowering melanin formation and relatively moderate cytotoxicity. The Micellar Arbutin cream was created using Urahexclusive ®'s micellar technology, and its ability to penetrate the skin and reduce cellular melanin was tested. The findings indicate that micellar arbutin cream enhanced both transport and cellular melanin suppression, indicating that micellar transdermal delivery may have potential use in treating hyper pigmentation (2016).

The metabolic disorder known as diabetes mellitus (DM), is characterized by hyperglycemia, is brought on by insulin deficiency and insulin resistance. It has been connected to peripheral and central nervous system issues, as well as neurological issues.

Peripheral neuronal deficits brought on by long term DM include axonal shrinkage, diminished nerve regeneration, reduced motor nerve conduction velocity, and faulty axonal transport. According to Osman et al. (2015),

Type II diabetes mellitus, hyperlipidemia, fatty liver, and cardiovascular illnesses are only a few of the consequences that are frequently connected to obesity and insulin resistance.

Additionally, according to recent estimations made on a global scale, the roughly 180 milli on people who had diabetes in 2000 will double in number by the year 2050 (Beaulieu et al.,2010)

The oxidation of l-tyrosine (monophenolase activity) mediated by mushroom tyrosinase was discovered to be inhibited by arbutin, hydroquinone-O-d-glucopyranoside . A catalytic amount of l-3,4-dihydroxyphenylalanine (l-DOPA) became available as a cofactor, which caused the oxidation of arbutin to accelerate. Nevertheless, arbutin itself was oxidized as a monophenol substrate at a remarkably slow pace. Monitoring oxygen consumption confirmed the outcome that was seen. If a cofactor is not present in the melanocytes, the depigmenting process of arbutin previously reported seems plausible. It is advantageous to combine with l-ascorbic acid, especially when oxygen is scarce. (John Wiley & Sons, 2004)

CHAPTER THREE

EXPERIMENTAL PART

Chapter Three:

Experimental Part

3.1 Materials & Reagents

Pear fruits were collected from Hebron city, Palestine, and other materials including ethanol 70%, acetic acid, hydrochloric acid 5% (HCl), monopotassium phosphate (KH_2PO_4), Disodium phosphate (Na_2HPO_4), distilled water, fructose, glucose, DPPH, methanol, trichloroacetic acid, potassium ferricyanide, Ferric chloride, Butylated hydroxytoluene (BHT), sodium bicarbonate, sodium nitrite, aluminum chloride, sodium hydroxide were purchased from sigma Aldrich. As well as, Quercetin standard with CAS No. (117-39-5) Q4951 and Gallic Acid standard (with CAS No. 149-91-7) G7384, Albumin from human serum (BSA) CAS No. (70024-90- 7) A9511, Folin & Ciocalteu′s phenol reagent F9252, were purchased from sigma Aldrich.

3.2 Instruments

The instruments used in the experiment are PERKIN-ElMER Lambda 5 UV/VIS Spectrophotometer, FLUROSKAN ASCENT FL, Analytical balance SHIMADZU ATx324 320g in Balances (S-841), Stuart Rotary Evaporator (RE 400) with Digital Water Bath (RE 400 DB), Ultrasonic Bath (Sonicator) IKON INDUTRIES (170VAC – 270VA),vortex, HPLC.

3.3 Sample preparation

Fresh Pyrus spp were collected from Hebron city, a Palestinian area in spring months of 2021 (exactly in late March). The fruits were peeled and then peel and flesh were separated of each other. The peel was made at a thickness of 1 mm with a peeler.

3.3.1 Extraction of pear peel:

A powdered sample (5.00 g) is placed in a 100-mL conical flask, and 100 mL of an extraction solution (water: ethanol) (v/v) =40:60 is added and was extracted by sonication at 24 ºC for half hour. The extract is filtered through a 0.45-mm membrane and then the extraction solution is evaporated at 50-57 C by rotary evaporation at al-Quds University, chemistry lab.

3.4 Fluorescence-based assay of the inhibition of AGE formation.

The method was performed for each sample as follows: (C. Harris et al., 2011):

a. Preparation of Incubation media :

1 A buffer with a pH of 7.4 was made, consisting of 100mM sodium phosphate monobasic monohydrate.

2 A stock solution of bovine serum albumin (BSA) (1 mg/ml) was made in a buffe r of sodium phosphate monobasic monohydrate (100 mM) (pH 7.4).

3 In 100mM of sodium phosphate monobasic monohydrate buffer, a 3- Stock solution of a combination of 100mM of glucose and 100mM of fructose was created (pH 7.4).

b. Preparation of samples and standard:

Each sample was dissolved in 2 ml DMSO then filtrated and dilute with 99% ethanol to prepare range of concentrations (200, 250, 300 and 400 ppm). Quercetin standard with same conc. of samples was prepared in 99% ethanol.

c. Test Samples:

1. Preparing extracts sample to test: 0.5 ml of each concentration samples was selected and mixed with 0.3 ml phosphate buffer pH 7.4, 0.1 ml solution sugar and 0.1 ml BSA solution.
2. Preparing negative control: 0.3 ml phosphate buffer, 0.1 ml solution sugar, 0.1 ml BSA solution & 0.5 ml 99% ethanol.
3. Preparing of positive control: 0.5 ml standard solution of each concentration was mixed with 0.3 ml phosphate buffer pH7.4, 0.1 ml solution sugar & 0.1 ml BSA solution.

All test samples were prepared in glass test tube & cover then incubated in incubator shaker at al-Quds university lab at 37 ºC for 7 days.

d. Fluorescence-based assay of the inhibition of AGE formation:

Using a fluorometer from the Nutrition and Health Research Institute of Al-Quds University, each sample's generation of fluorescent antiglycation end products (AGEs) was quantitatively evaluated after 7 days of incubation. The excitation and emission wavelengths were 455 nm and 375 nm, respectively. To eliminate baseline fluorescence, the fluorescence measurements for the experimental treatment—consisting of BSA, sugar, and either an extract or a pure standard—and the negative control were blanked against the corresponding extract blanks, phosphate buffer, and BSA. The percentage of inhibition of AGE production was calculated using the corrected fluorescence readings (F) for the experimental treatments (F experimental corrected) and for the negative control (F negative control).

$$\% \text{ inhibition} = \frac{(\text{F negative control} - \text{ F experimentalcorrected })}{\text{Fnegative control}} * 100\ \%$$

3.5 Free radical scavenging activity using DPPH (DPPH)

The antioxidant strength of extracts was assessed as follows Different concentrations of the sample (350,300,250,200, and 150 ppm) were used, and from each concentration, 0.5 ml of extract was taken and mixed with 1.5 ml of DPPH (0.04g/100 mL 80 percent methanol), 3 mL of 96 percent ethanol, and a negative control of 1.5 mL of DPPH and 3.5 mL of 96 percent ethanol, and the mixture was then incubated in At 517 nm, the absorbance was measured. The results were calculated as a percentage of control inhibition. Using the following equation, the samples' percentage of DPPH inhibition was determined:

$$\% \, Inhibition = \frac{A^{\circ} - A}{A^{\circ}} * 100\ ,$$

Where A° is the absorbance of a solution of 100-µL methanol 95% and 3.9 mL of DPPH at 517 nm and A is the absorbance of the sample extract at 517 nm. (Jothy, Subramanion L., et al2011).

3.6 Total phenolic content (Folin-Ciocalteau assay):

The total phenol of the was obtained by Folin-Ciocalteau reagents .The conc. of the extract was prepared by dissolving in 2 ml 99% DMSO and diluted with distilled water to prepare sequentially (100, 100, 200 & 150 ppm), 0.5 ml of each extract with 2.5 ml of Follin reagent (10 %, v\v), 2.5 ml sodium carbonate (7.5 %, w\v) then incubated for 30 min in the dark at

room temperature, the absorbance was measured at 760 nm. Different concentration of gallic acid standard (20-110 ppm) for calibration curve. Results were shown as mg Gallic acid equivalents (GAE)\g sample. (Uddin, Md, et al).

3.7 Total flavonoid content:

The extract's total flavonoid content was assessed .2000 ppm of the extract was prepared. Next, a sample of 0.5 ml of the extract was combined with 1.5 ml of 95% ethanol, 0.1 ml of aluminum chloride (10% w/v), 0.1 ml of 1M sodium acetate, and 2.8 ml of distilled water. This mixture was then incubated for 30 min at room temperature and in the dark. 415 nm was used to measure the absorbance. For the calibration curve, different quercetin concentrations (ranging from 2.5 to 200 ppm) were generated. (Kim, et al 2003)

3.8. HPLC analysis

3.8.1 HPLC Instrumentation systems

The analytical HPLC is Waters Alliance (e2695 separations module), quipped with 2998 Photo diode Array (PDA). Data acquisition and control were carried out using Empower 3 chromatography data software (Waters, Germany).

3.8.2 Chromatographic conditions

The HPLC analytical experiments of the crude extracts were run on ODS column of Waters (XBridge, 4.6 ID x 150 mm, 5 μm). The mobile phase is a mixture of 0.5% acetic acid solution (A) and acetonitrile (B) ran in a linear gradient mode. The start was a 100% (A) that descended to 70% (A) in 15 minutes. Then to 40% (A) in 5 minutes and finally to 10% (A) in 4 minutes and stayed there for 6 minutes. The HPLC system was equilibrated for 5 minutes with the initial acidic water mobile phase (100 % A) before injecting the next sample. All the samples were filtered with a 0.45 μm PTFE filter. The wavelengths was 250. The flow rate was 1 ml/min. Injection volume was 20 μl and the column temperature was set at 25◦C.

3.8.3 Standard of arbutin preparation

Standard of arbutin at a concentration of 0.1 mg per mL was prepared in 50% acetonitrile, and 20 microliter was injected into the HPLC using the method described in the previous section.

3.8.4 Sample preparation

The plant extracts were filtered using suction filtration, and then the solvents were evaporated under reduced pressure at 40 C using Rotary evaporator. The resulting crude extracts were dissolved in 80% ethanol at a concentration of 2 mg/mL, and 20 µL were injected into the HPLC chromatograph, and then analyzed to determine their arbutin content.

3.9. Construction of ternary phase diagram:

The water titration method was used to create the pseudo ternary phase diagramsmade up o f a mixture of IPM as the oil phase, pear peel extract as water phase , Tween 20 as surfactant, and ethanol as co-surfactant with various HLB values.

Procedure:

1) At room temperature, samples of IPM and nonionic surfactant (Tween20) were placed in 10 ml glass test tubes with screw lids at various weight ratios as shown in Table (3.1).

2) Each sample was mechanically shaken for 1 minute on the vortex.

3) A drop by drop titration with water phase, which is a combination of pear peel extract and co-surfactant (ethanol) with a ratio of 1:1, was injected into each test tube.

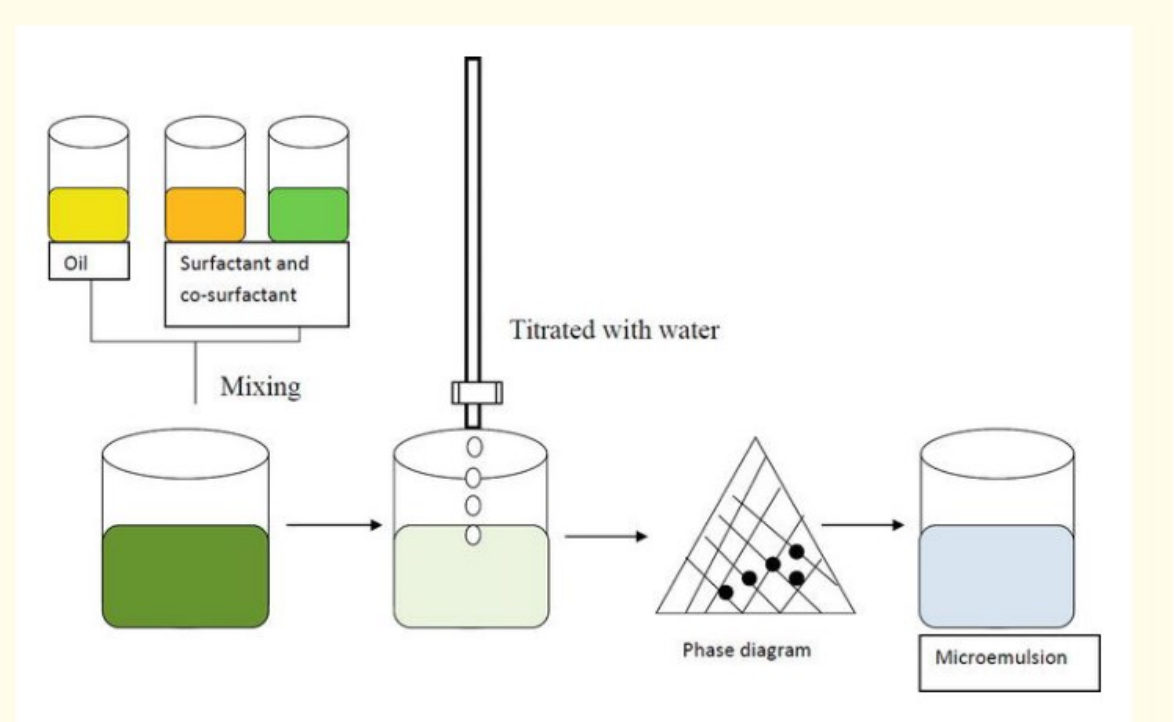


Figure (3.1): titration method to prepare a micro-emulsion (Abhishek,2019)

4) Due to the high viscosity of the surfactant and oil, vigorous mechanical shaking for 5 minutes on the vortex is required to ensure a homogeneous dispersion.

5) Rest for 24 hours to allow for equilibrium before the next addition of water and analysis.

Table 3.1 the ratio between oil extract and nonionic surfactant

Weight ratio	1:9	2:8	3:7	4:6	5:5	6:4	7:3	8:2	9:1
Surfactant(tween20)	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**
Oil(IPM)	**0.9**	**0.8**	**0.7**	**0.6**	**0.5**	**0.4**	**0.3**	**0.2**	**0.1**

3.9.1. Determination of phase behavior:

Anisotropy tests and visual observations of the behavior of oil, water, surfactant, and co-surfactant emulsions will be conducted.

1) Visual observation: Once the emulsion is formed, the first step in figuring out the phase behavior is to visually check it. Depending on how much water is added, different results will be obtained in terms of color, texture, and transparency.

Transparent, single-phase, low viscosity mixtures are known as microemulsions.

2) Anisotropy can be detected using cross polarizers.

Microemulsion systems were defined as clear, isotropic, one-phase systems.

3)Draw the phase diagram using ternary plotting program.

CHAPTER FOUR

RESULTS & DISCUSSIONS

Chapter 4:

Results and Discussion

4.1. Extraction yield

The weight of dried extracts was obtained after evaporation of the solvent for 24 hours at room temperature 2.5g per 5.1113g pear peel powder this, that means the percentage yield of extraction was 50% it is close to the literature value in one of the studies that was 59.22% (Kim et al 2013)

4.1.1 Selection of the extraction Solvent and the condition for extraction:

An essential analytical stage in the creation of the method for the qualitative and quantitative analysis of the biologically active chemicals in raw plant material is the choice of the solvent and the extraction conditions. One of the key variables in predicting the qualitative and quantitative content of the recovered phenolic compounds is the extraction solvent. The pear fruit samples produced the highest amount of phenolic compounds when they were extracted for 15 minutes in an ultrasonic bath using 70% ethanol, according to the studies' results for choosing the extraction method. Based on these findings, these extraction conditions were chosen for additional research. Therefore, ethanol 70% is utilized as a solvent and extract by sonication method. (Liaudanskas, et al 2017)

Figure 4.1: The dried pear peel

figure 4.2: The ethanoic pear peel extract by sonication method

4.2 Antiglycation End Products (AGEs) Assay

The test demonstrated that pear peel extract was found to have beneficial effects on the decrease of AGEs by utilizing a fluorometer to measure the fluorescence or light released by fluorescing glycation products at a particular wavelength of excitation and emission. This inhibition was expected because of the pear peel enrich with arbutin that is possessed an in vitro antiglycation activity (arome,2005) Figure (4.3) illustrates how the percentage of AGE inhibition rose linearly as concentration decreased, going from 18 % at the highest concentration of 350 ppm to 16.53% at the lower concentration of 250 ppm.

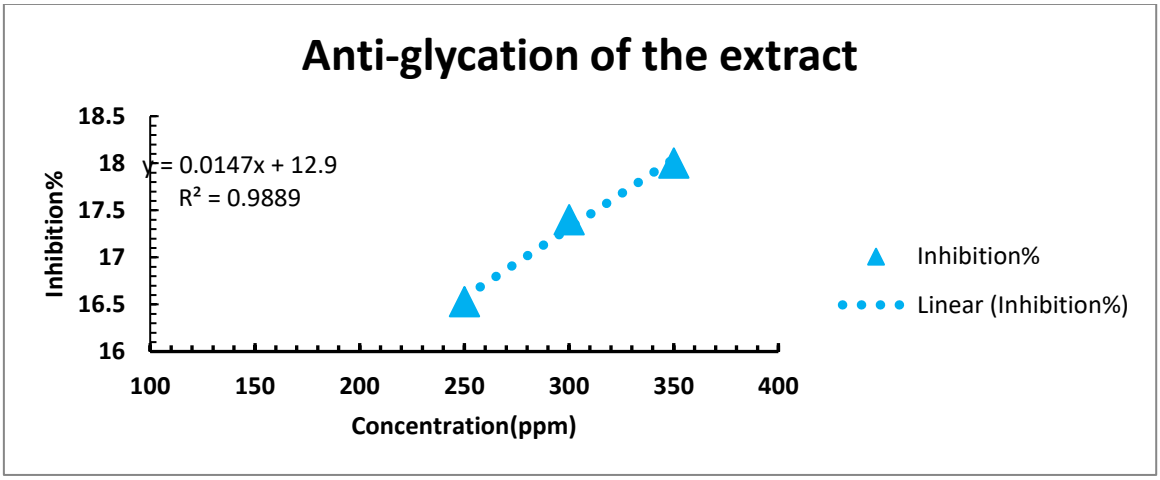


Figure (4.3): Concentration dependent effects of pear peel extract on in vitro formation of fluorescent AGEs.

4.2. Total phenolic content

Using the Folin- Ciocalteu reagent, the total phenolic content of the pear peel was estimated to be 464.4mg of gallic acid equivalents (GAE) per gram of plant extrac in this study. The standard curve of gallic acid was used to compute the total phenolic contents of the test fractions. In one of the studies which is taken from the packman and Yali pears The total amount of phenol of Extracts of the pear peels were 62.04 1,044 mg GAE/g and 57.500.817 mg GAE/g, respectively Using ethanol 70% as a solvent, the maceration method was used to obtain the extracts,(Vinda et al 2019) .According to the quantitative results in the pear peel in our study is higher, and that may be for which is the difference in the method of extraction in our study. The extraction was done by the sonication method, and the reason may be the difference in the geographical spot of the plant.

4.4 Total flavonoids content:

The content of total Flavonoids in the extract of *pear peel* was 10.68 mg/QUW.while flavonoid contents in Packham's pear peel and Yali's pear peel are 7.40% for Packham's pears and 6.03% for Yali's pears (Vinda et al 2019)

4.5 Anti-oxidant assay:

By using the PDDH Assay, the free radical scavenging ability (absorbance) of the pear peel extract was measured and expressed as a percentage of its scavenging effect. The scavenging effect of pear peel extract ranged from 32% at the lowest concentration ratio of 150 ppm to 39.7% at the highest concentration ratio of 300 ppm.That indicate when the concentration of the extract is increasing the antioxidant activity is increasing due to the pear peel enrich with vital antioxidant. Figure (4.5)

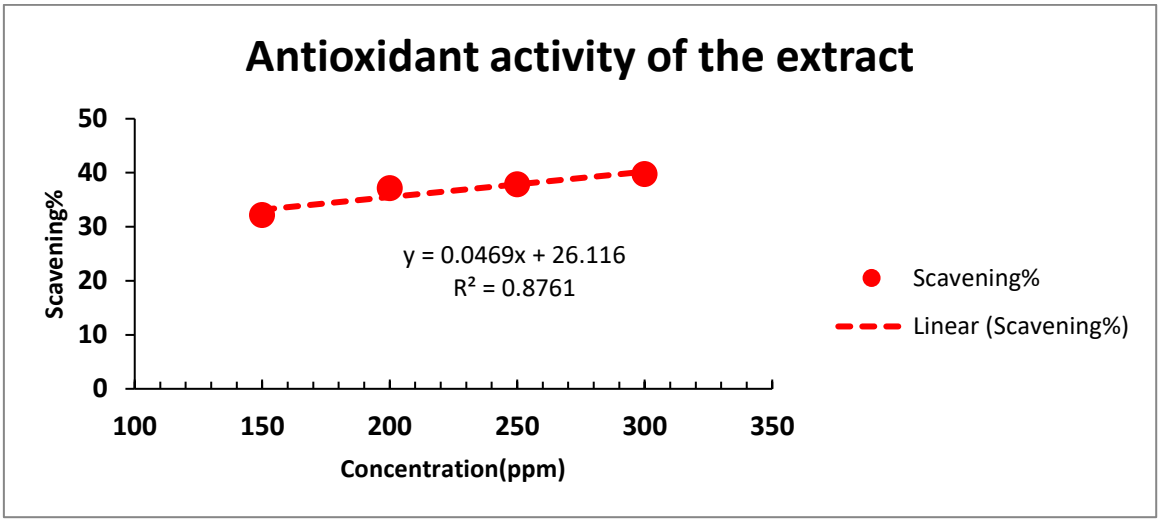


Figure 4.4: DPPH Scavenging activity of pear peel extract.

4.6 HPLC analysis

The pear peel extract sample's HPLC chromatogram revealed arbutin to be present at a rete ntion time of 3.0 minutes. The retention time of arbutin in the standard solution of arbutin, which was evaluated under the identical conditions, was used to identify the arbutin peak.fi g (4.6). As shown in the figure, the quantity of pear peel arbutin is high. This indicates that that stability conditions of arbutin stability under the control and didn’t analysis.

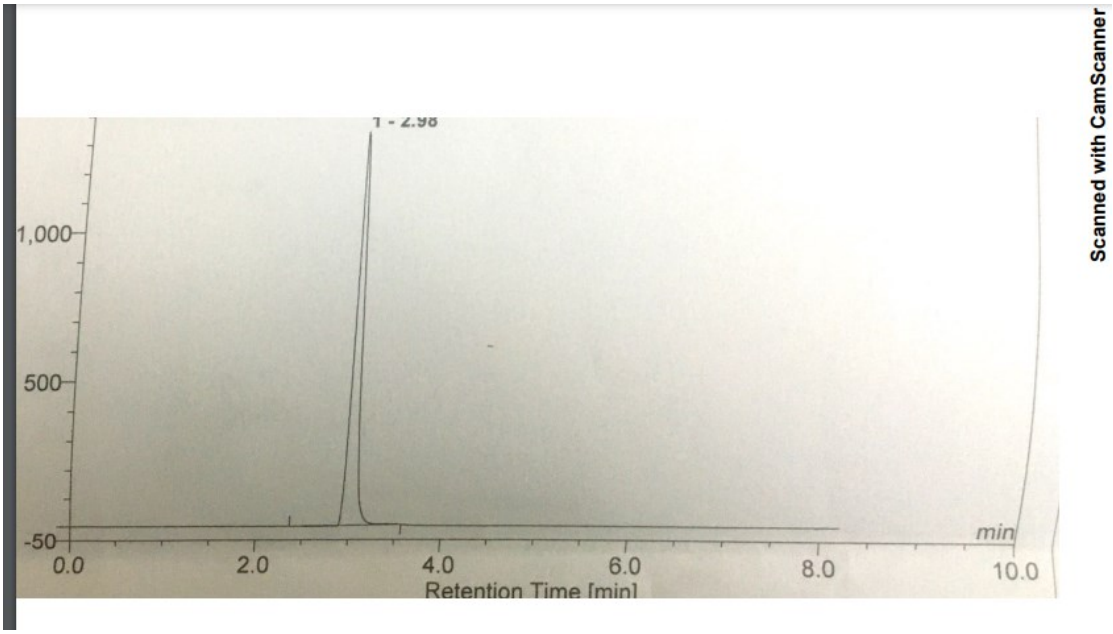


Figure 4.5: HPLC chromatogram for standard arbutin

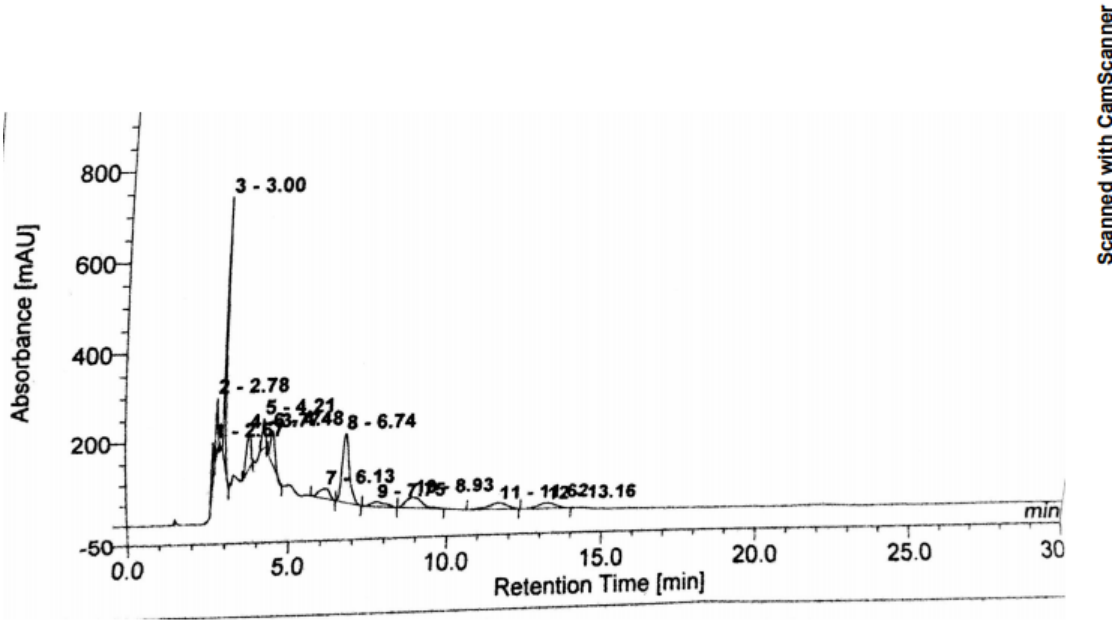


Figure 4.6: HPLC chromatogram for pear peel extract using the method described in section 3.9

4.7 Constructing of Phase behavior

Figure 4.8 shows the pseudo-ternary phase diagram of the microemulsion system, which consists of IPM as the oil phase, Tween 20 as the surfactant and extract/ethanol (co-surfactant) with a ratio of (1:1) as the water phase. The water didn't used as the water phase to maintain the stability of arbutin in the extract. Ethanol 95% is used to enhance the solubility. IPM is used as oil phase.

On the phase diagram, the percentages by weight of the extract / co-surfactant phase, surfactant, and oil that produced a three regions which are 1Φ, 2Φ and 3Φ.

1Φ: is a one phase region, it's a microemulsion region; it is a clear, transparent and isotropic region.

2Φ: is a two phase's region, it's an emulsion region, is a cloudy region.

3Φ: is a three phases region.

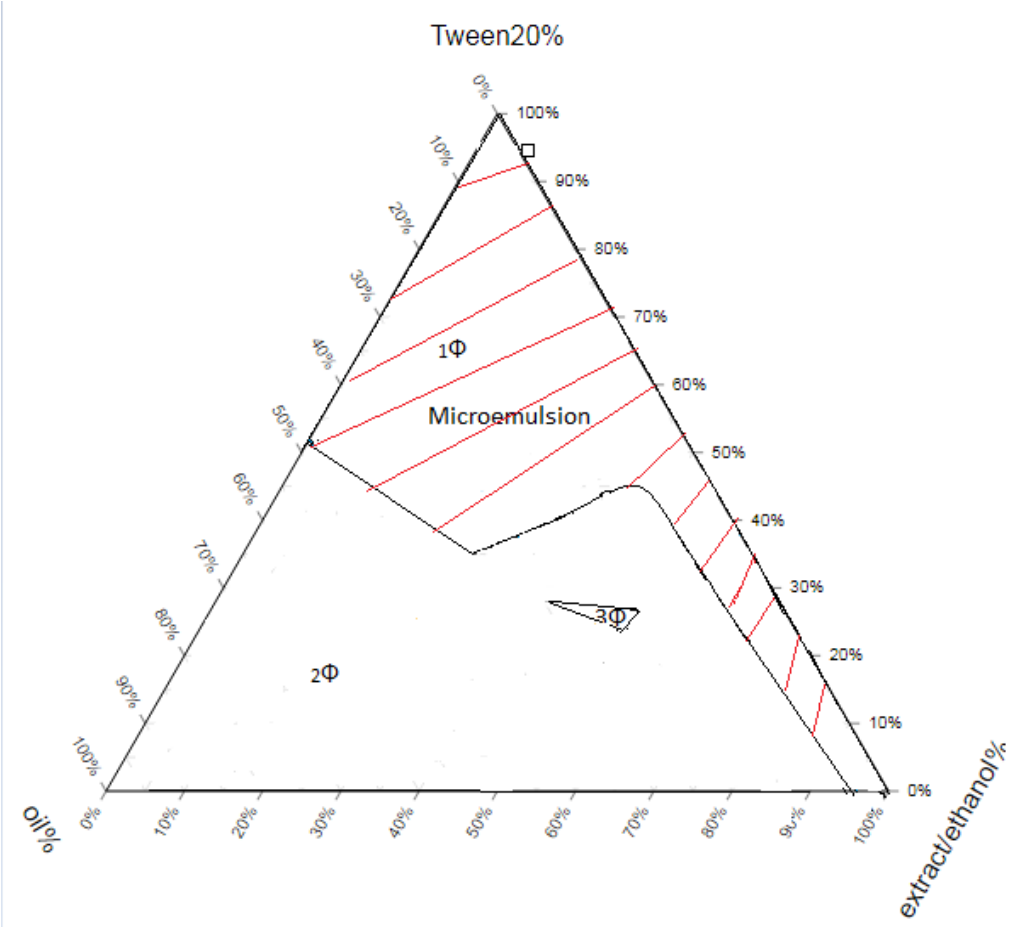


Figure (4.8): Psedo-ternary phase of pear peel extract

The micro-emulsion region is the largest region in the psedo-ternary phase diagram, so the choosing the micro-emulsion technique to development the delivery of the components of the peel extract was a good choice figure 4.8

Figure 4.9 illustrates how to make a microemulsion with pear peel extract it must be emphasized that the microemulsion encapsulated extract is expected to have 100% efficiency.

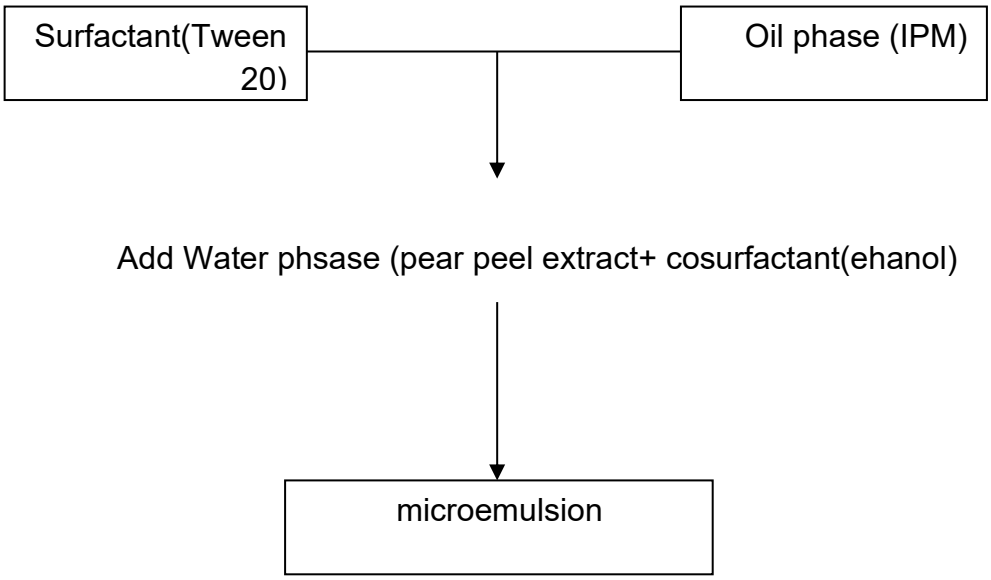


Figure (4.9): preparation of microemulsion by the pear peel extract.

CHAPTER FIVE

CONCLUSION & FUTURE WORK

Chapter five:

Conclusion & future work:

5.1. Conclusion:

The successful microemulsion system is shown in this work as a promising vehicle for topical distribution of the natural extract. The current investigation demonstrated the effectiveness of biochemical pear peel extracts in preventing the formation of glycation end products, and the presence of antioxidant components such as phenol and flavonoids. This discovery implies the potential use of pear peel extract as a food supplement because it is inexpensive, safe and effective at preventing the production of glycations and, thus, the development of diabetes problems. According to an HPLC study, the pear peel has a high amount of arbutin, making it a natural bleaching agent.We could construct a psedo-ternary phase with pear peel extract. This application enhance the bioactivity and the permeability of arbutin's pear peel into skin and thus overcomes the low permeability of arbutin to skin due to the high hydrophobicity of arbutin.

5.2 Future work:

1. Isolation of arbutin of pear peel by HPLC with high recovery and purity and using it in cosmetic as whitening agent.

2. Development and characterization of cosmetic micro-emulsion containing arbutin's pear peel.

3. Development of supplement tablets or capsules containing pear peel extract for diabetes disease.

4. Isolation of total phenols, antioxidants and flavanoids from pear peels and using them as antiaging agents in cosmetics and nutritional supplements.

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APPENDIX

Appendix

Appendix (a): Anti-glycation results for samples & positive control:

Negative Control Samples	
Fluorescence Response	Average Fluorescence Response
1.664	1.5
1.658	
1.671	

Pear peel samples					
concentration (ppm)	Fluorescence Response	% of inhibition	Average % of inhibition	Standard Deviation	Standard error
250	1.218	18	17.3	0.7391	0.4267
300	1.238	17.4			
350	1.252	16.53			

Appendix (b): Total phenols content results for all tested samples

The standard curve of the Gallic Acid gives the following liner equation: Y=0.0097X-0.1555					
Extract A 0.0001g/ml	Absorbance	mg GAE \ g dry extract	Average mg GAE \ g dry extract	Standard deviation	Standard error
The sample	0.301	470.6	464.4	5.7294	3.3079
	0.290	459.28			
	0.294	463.4			

Appendix (c): Total Flavonoids content results for all tested samples

The standard curve of the Quarectin gives the following liner equation: y = 0.0097x + 0.0168					
Extract A 0.002mg/ml	Absorbance	mg QE\ g dry extract	Average mg QE \ g dry extract	Standard deviation	Standard error
The sample	0.214	10.17	13.9099	5.4958	3.1730
	0.236	11.34			
	0.215	20.22			

Appendix (d): DPPH result for all tested samples

Control	Absorbance	Average absorbance	Standard deviation	Standard error	
Reading 1	1.940	1.94	0.0007	0	
Reading 2	1.940				
Absorbance of control (400µL of ethanol 96% and 1.5 mL of DPPH) = 1.299					
Concentration (ppm)	scavenging capacity (Absorbance)	Scavenging effect %	Average Scavenging effect %	Standard deviation	Standard error
150	1.238	36.21	32.11	5.8053	4.1050
	1.396	28			
200	1.320	32	37.1	7.2125	5.1000
	1.121	42.2			
250	1.283	33.86	37.8	5.4518	3.9450
	1.130	41.75			
300	0.986	49.17	39.7	13.4138	9.4850
	1.354	30.20			

مستحلب دقيق يحتوي على جزيئات أربوتين النانوية الطبيعية

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الملخص:

يتم إنتاج قشور الفاكهة بكميات هائلة كمنتج ثانوي من منتج الكمثرى لإنتاج أربوتين ، إلى جانب حمض الكلوروجينيك والروتين ، وهو أحد المواد الكيميائية الفينولية الموجودة في ثمار الكمثرى وقشورها. في هذا البحث ، ركزنا على نظير الكمثرى لأن قشر الكمثرى هو نفايات بيولوجية ومنتج ثانوي ويمكننا تحقيق العديد من الأهداف. أولاً ، يتم استخلاص قشر الكمثرى بطريقة الصوتنة حيث استخدمنا الإيثانول بنسبة 70٪ كمذيب ، وتم الحصول على 51٪ من المستخلص. ثانيًا ، ظهرت كمية أربوتين في المستخلص الطبيعي بكمية عالية من أربوتين وفقًا لتحليل كروماتوجرافيا السائل عالي الأداء (HPLC). ثالثًا ، تم تحديد إنتاج المستخلص المضاد للجلوكوز بنسبة 18٪ عند 350 جزء في المليون من المستخلص باستخدام اختبار الألبومين المصل الجلوكوز البقري (BSA) في المختبر. ثم تم تقييم التأثيرات المضادة للأكسدة بين 32٪ إلى 39.7٪ بطريقة الكسح 2 ، 2- diphenyl-1-picrylhydrazyl (DPPH)). تم استخدام اختبار Folin-Ciocalteau لتحديد المحتوى الفينولي الكلي (TPC) والذي كان 64.4 مجم / GAE ، واستخدم اختبار قياس الألوان لتحديد محتوى الفلافونويد الكلي (TFC) الذي كان 13.9099 مجم / QE. أخيرًا ، تم تحميل مستخلص الكمثرى على مستحلب دقيق ، وهو مستقر ديناميكي حراري لزيادة التوافر البيولوجي والنفاذية عبر الجلد. تم تحضير مخطط الطور الثلاثي الزائف بطريقة المعايرة ورسمه لتحديد مناطق الاستحلاب الدقيق. يتكون المستحلب الدقيق من Isopropylmyristate (IPM) كمرحلة زيت ، Polysorbate 20 (Tween20) كمادة خافض للتوتر السطحي ، و ehanol كمرحلة خافض للتوتر السطحي ، ومستخلص قشر الكمثرى كمرحلة مائية. ظهرت ثلاث مناطق في مخطط الطور الثلاثي pesdo وهي منطقة المستحلب الدقيق ومنطقة المستحلب ومنطقة المراحل الثلاث. كانت منطقة المستحلب الدقيق هي الأعلى.