LAMPIRAN

PENETAPAN KADAR FLAVONOID TOTAL EKSTRAK ETANOL KULIT BUAH ALPUKAT *(Persea americana* Mill.*)* DENGAN METODE SPEKTROFOTOMETRI UV-VIS

Aminah[1], Nurhayati Tomayahu, Zainal Abidin
Fakultas Farmasi, Universitas Muslim Indonesia
[1]aminah.aminah@umi.ac.id

ABSTRACT

Avocado plant (Persea americana Mill.), belongs to Lauraceae family which have medicinal properties. Avocado peel contains flavonoid compounds that can be used to protect and reduce the skin damage against UV rays. This study aimed to determine the total flavonoids content of the ethanol extract of the avocado peel using UV-Vis spectrophotometry. The ethanol extract of avocado peel was obtained by macenation with ethanol. The qualitative analysis used $FeCl_3$ by forming green complex. The determination of total flavonoids content is conducted based on $AlCl_3$ method with total flavonoids expressed in QE (Quercetin equivalent) at the maximum wavelength of 435 nm. The result showed that the average content of flavonoid total is 4.0122 mgQE/g extract.

Keywords: *Flavonoid Total, Caucasian avocado (Persea americana Mill.), UV-Vis spectrophotometry*

I. PENDAHULUAN

Indonesia merupakan negara yang memiliki kekayaan alam dengan berbagai jenis tanaman yang dapat berkhasiat sebagai obat tradisional. Obat tradisional semakin banyak diminati oleh masyarakat karena bahan nabatinya mudah didapat, mudah diracik dan harganya terjangkau, sehingga bahan yang digunakan harus ditingkatkan mutu dan kualitasnya sesuai dengan kebutuhan masyarakat.

Salah satu tumbuhan yang menarik untuk diteliti adalah buah alpukat dari family *Lauraceae* yang merupakan tanaman yang dapat berkhasiat sebagai obat, tanaman ini dapat tumbuh di daerah tropis dan subtropis (Katja, Suryanto & Wehantouw 2009, h. 58).

Alpukat secara empiris berkhasiat mengobati penyakit seperti sariawan, kencing batu, sakit gigi, muka kering, bengkak karena peradangan dan juga kencing manis (Katja, Suryanto & Wehantouw 2009, h. 58). Kulit alpukat mengandung senyawa flavonoid yang dapat digunakan untuk melindungi kulit terhadap sinar UV atau mampu mengurangi kerusakan kulit, karena senyawa ini bekerja sebagai bahan aktif tabir surya (Mokodompit, Edy dan Wiyono 2013, h. 85).

Flavonoid merupakan salah satu senyawa golongan fenol alam terbesar yang terdapat dalam semua tumbuhan hijau (Markham, K.R 1988). Menurut (Pourmorad, F 2006, h. 1143) mengemukkan bahwa salah satu golongan senyawa polifenol ini diketahui memiliki sifat sebagai penangkap radikal bebas, penghambat enzim hidrolisis, oksidatif, dan juga bekerja sebagai antiinflamasi.

Berdasarkan uraian tersebut, maka perlu dilakukan penelitian yang lebih intensif mengenai pengujian kadar flavonoid total dari ekstrak etanol kulit buah alpukat *(Persea americana* Mill.*)*, sehingga potensi tumbuhan ini sebagai bahan baku obat untuk pencegahan maupun pengobatan berbagai penyakit dapat lebih dikembangkan dengan maksimal.

II. METODE PENELITIAN

A. Pengambilan dan Pengolahan Sampel

Pengambilan Sampel buah alpukat *(Persea americana* Mill.*)* dilakukan pada pagi hari sekitar pukul 10.00 WITA di Malino, Sulawesi selatan. Kemudian disortasi basah untuk menghilangkan tanah dan pengotor lainnya yang masih menempel pada sampel. Kemudian buah alpukat dipisahkan dari daging buah dan kulit buahnya lalu di bersihkan. Kulit buah alpukat *(Persea americana* Mill*.)* yang telah dibersihkan dilakukan pengubahan bentuk dengan cara dipotong-potong kecil, selanjutnya dikeringkan dengan cara diangin-anginkan selama beberapa hari pada udara terbuka dengan tidak terkena sinar matahari langsung. Setelah kering sampel ditimbang dan dicatat berat keringnya kemudian diserbukkan setelah itu ditimbang kembali berat sampel serbuk yang di peroleh (Dahlia & Ahmad 2016, h. 16).

B. Proses ekstraksi kulit alpukat *(Persea americana* Mill.*)*

Sebanyak 50 gram sampel kulit buah alpukat (*Persea americana* Mill.) dimasukkan kedalam wadah maserasi. Kemudian ditambahkan dengan etanol 96% 200 mL sampai seluruh sampel terendam, kemudian ditutup dan dibiarkan selama 24 jam. Maserat disaring dengan menggunakan kertas saring. Filtrat diperoleh melalui penyaringan dengan corong, kemudian ampas dimaserasi kembali dengan etanol 96% 200 mL, sehingga filtrat hampir tidak berwarna.

Semua filtrat disatukan dan dipekatkan dengan menggunakan rotavapor sampai tidak ada lagi cairan yang menetes sehingga diperoleh ekstrak etanol kulit buah alpukat (*Persea americana* Mill.). Ekstrak kental kulit buah alpukat (*Persea americana* Mill.) yang didapatkan digunakan untuk dianalisis lebih lanjut (Gustandy, M dan Soegihardjo, C.J 2016).

C. Analisis Kuantitatif Kandungan Flavonoid

diperoleh nilai rata-rata absorbansi (Stankovic, M.S., 2011, h. 65).

III. HASIL DAN PEMBAHASAN

Tumbuhan alpukat *(Persea americana* Mill.*)* yang digunakan dalam penelitian ini yaitu hanya pada bagian kulit buahnya. Kulit buah alpukat *(Persea americana* Mill.*)* dapat digunakan sebagai bahan aktif tabir surya yaitu untuk melindungi kulit terhadap sinar

C. Analisis Kuantitatif Kandungan Flavonoid

Sebanyak 1 mg ekstrak etanol kulit buah alpukat (*Persea americana* Mill.) ditambahkan dengan 2 tetes $FeCl_3$. Terbentuknya warna hijau atau hijau biru menunjukkan adanya senyawa flavonoid dalam bahan (Harborne, J.B 1987).

D. Analisis Kualitatif Kandungan Flavonoid

1. Penentuan panjang gelombang maksimum (λ_{maks}) kuersetin

Penentuan panjang gelombang maksimum kuersetin dilakukan dengan *running* larutan kuersetin pada range panjang gelombang 400 - 450 nm. Hasil *running* menunjukkan panjang gelombang maksimum standar baku kuarsetin berada pada panjang gelombang 435 nm. Panjang gelombang maksimum tersebut yang digunakan untuk mengukur serapan dari sampel ekstrak etanol kulit buah alpukat *(Persea americana* Mill.*)*.

2. Pembuatan kurva standar kuarsetin

Ditimbang sebanyak 25 mg baku standar kuersetin dan dilarutkan dalam 25 mL etanol. Larutan stok dipipet sebayak 1 mL dan dicukupkan volumenya sampai 10 mL dengan etanol sehingga diperoleh konsentrasi 100 ppm. Dari larutan standar kuersetin 100 ppm, kemudian dibuat beberapa konsentrasi yaitu 6 ppm, 8 ppm, 10 ppm, 12 ppm dan 14 ppm. Dari masing-masing konsentrasi larutan standar kuersetin dipipet 1 mL. Kemudian ditambahkan 1 mL $AlCl_3$ 2% dan 1 mL kalium asetat 120 mM. Sampel diinkubasi selama satu jam pada suhu kamar. Absorbansi ditentukan menggunakan metode spektrofotometri UV-Vis pada panjang gelombang maksimum 435 nm (Stankovic, M.S., 2011, h. 65).

3. Penetapan kadar flavonoid total ekstrak etanol kulit buah alpukat *(Persea americana* Mill.*)*

Ditimbang 15 mg ekstrak, dilarutkan dalam 10 mL etanol, sehingga diperoleh konsentrasi 1500 ppm. Dari larutan tersebut dipipet 1 mL kemudian ditambahkan 1 mL larutan $AlCl_3$ 2% dan 1 mL kalium asetat 120 mM. Sampel diinkubasi selama satu jam pada suhu kamar. Absorbansi ditentukan menggunakan metode spektrofotometri UV-Vis pada panjang gelombang maksimum 435 nm. Sampel dibuat dalam tiga replikasi untuk setiap analisis dan

tabir surya yaitu untuk melindungi kulit terhadap sinar UV atau mampu mengurangi kerusakan kulit, karena mengandung senyawa flavonoid (Mokodompit, Edy dan Wiyono 2013, h. 85).

Flavonoid hampir terdapat pada semua bagian tumbuhan termasuk buah, akar, daun, dan kulit luar batang. Flavonoid merupakan senyawa alam yang berpotensi sebagai antioksidan yang dapat menangkal radikal bebas yang berperan pada timbulnya penyakit degeneratif melalui mekanisme perusakan sistem imunitas tubuh, oksidasi lipid dan protein (Rais, I.R 2015, h. 103).

Pada penelitian ini buah alpukat yang digunakan diperoleh dari Malino, Sulawesi selatan. Kotoran ataupun serangga yang menempel pada buah alpukat harus dibersihkan karena dapat mengganggu proses dan hasil ekstraksi. Kulit buah alpukat *(Persea americana* Mill*)* dilakukan pengubahan bentuk dengan cara dipotong-potong kecil untuk mempercepat proses pengeringan. Proses pengeringan ini dimaksudkan untuk mengurangi kadar air yang terdapat pada sampel, sehingga dapat mencegah pembusukan oleh bakteri.

Proses ekstraksi dilakukan bertujuan untuk mengambil senyawa kimia yang terkandung dalam sampel. Prinsip ekstraksi didasarkan pada perpindahan masa komponen zat yang terlarut ke dalam pelarut sehingga terjadi perpindahan pada lapisan antar muka dan berdifusi masuk ke dalam pelarut (Harborne, J.B 1987). Pelarut yang digunakan pada penelitian ini adalah etanol 96% sebagai pelarut polar. Dalam hal penyarian, etanol memiliki kelebihan dibandingkan dengan air dan metanol. Senyawa kimia yang mampu disari dengan etanol lebih banyak dari pada penyari metanol dan air (Azizah dan Salamah 2013, h. 24).

Untuk mendapatkan senyawa kimia yang diinginkan digunakan metode ekstraksi yang merupakan metode penyarian zat berkhasiat atau zat aktif dari bagian tanaman dengan menggunakan pelarut yang sesuai (Yuliani & Satuhu, 2012).

Metode ekstraksi yang digunakan pada penelitian ini adalah maserasi, karena metode ini lebih sederhana, mudah dan tanpa pemanasan. Karena jika menggunakan pemanasan dapat membuat kadar flavonoid berkurang. Proses maserasi menggunakan 3 replikasi dengan etanol 96% 200 mL selama 24 jam. Penambahan pelarut etanol dilakukan sampai 3 kali proses ekstraksi.

Ekstrak yang diperoleh dipekatkan dengan rotavapor sampai diperoleh ekstrak kental yang berwarna hijau tua. Kemudian dilakukan perhitungan rendamen, sehingga diperoleh rata-rata persen rendamen yaitu 17,28 %. Penentuan rendamen ini berfungsi untuk mengetahui kadar metabolit sekunder yang terbawa oleh pelarut namun tidak dapat menentukan jenis senyawa yang terbawa oleh pelarut (Ahmad, Juwita dan Malik 2016, h. 6). Dapat dilihat pada tabel 1.

Tabel 1. Hasil ekstrak etanol dari kulit buah alpukat *(Persea americana* Mill.*)*

Jenis pelarut	Volume pelarut	Berat sampel(g)	Berat ekstrak(g)	% rendamen	Rata-rata % rendamen
Etanol 96%	200 mL	50	8,9689	17,9378	
	200 mL	50	8,5552	17,1104	17,28

baku, untuk membuat kurva baku terlebih dahulu dibuat beberapa deret konsentrasi untuk mendapatkan persamaan linear yang dapat digunakan untuk menghitung persen kadar. Digunakan kuarsetin sebagai larutan standar karena kuersetin merupakan flavonoid golongan flavonol yang mempunyai gugus keto pada C-4 dan memiliki gugus hidroksil pada atom C-3 atau C-5 yang bertetangga dari flavon dan flavonol (Azizah dan Faramayuda 2014, h. 48). Pengukuran serapan panjang gelombang maksimum dilakukan *running* dari panjang gelombang 400 – 450 nm. Hasil *running* menunjukkan panjang gelombang maksimum standar baku kuarsetin berada pada panjang gelombang 435 nm. Panjang gelombang maksimum tersebut yang digunakan untuk mengukur serapan dari sampel ekstrak etanol kulit buah alpukat *(Persea americana* Mill.*)*. Dapat dilihat pada tabel 3.

Tabel 1. Hasil ekstrak etanol dari kulit buah alpukat *(Persea americana* Mill.*)*

Jenis pelarut	Volume pelarut	Berat sampel(g)	Berat ekstrak(g)	% rendamen	Rata-rata % rendamen
Etanol 96%	200 mL	50	8,9689	17,9378	17,28
	200 mL	50	8,5552	17,1104	
	200 mL	50	8,3959	16,7918	

Analisis kualitatif dilakukan untuk mengetahui komponen kimia pada tumbuhan dengan menggunakan reagen besi (III) klorida ($FeCl_3$). Diamati perubahan warna yang terbentuk yaitu warna hijau (Harborne, J.B 1987). Hasil identifikasi menunjukkan ekstrak etanol kulit buah alpukat *(Persea americana* Mill.*)* positif mengandung flavonoid yang dapat dilihat pada tabel 2.

Tabel 2. Hasil analisis kualitatif ekstrak etanol kulit buah alpukat *(Persea americana* Mill.*)*

Sampel	Pereaksi	Warna	Ket
Ekstrak etanol kulit buah alpukat *(Persea americana* Mill.*)*	$FeCl_3$	Hijau	(+)

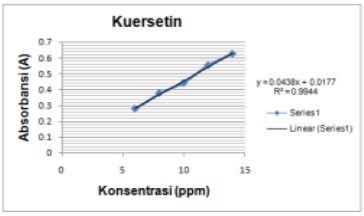
Analisis kuantitatif senyawa flavonoid total dengan menggunakan spektrofotometri UV-Vis dilakukan untuk mengetahui seberapa besar kadar flavonoid total yang terkandung pada ekstrak etanol kulit buah alpukat *(Persea americana* Mill.*)*. Analisis flavonoid dilakukan dengan menggunakan Spektrofotometri UV-Vis karena flavonoid mengandung sistem aromatik yang terkonjugasi sehingga menunjukkan pita serapan kuat pada daerah spektrum sinar ultraviolet dan spektrum sinar tampak (Harborne, J.B 1987).

Pada penelitian ini untuk menentukan kadar flavonoid total pada sampel digunakan kuarsetin sebagai larutan standar dengan deret konsentrasi 6, 8, 10, 12 dan 14 ppm. Digunakan deret konsentrasi karena metode yang di pakai dalam menentukan kadar adalah metode yang menggunakan persamaan kurva

Pengukuran serapan panjang gelombang maksimum dilakukan *running* dari panjang gelombang 400 – 450 nm. Hasil *running* menunjukkan panjang gelombang maksimum standar baku kuarsetin berada pada panjang gelombang 435 nm. Panjang gelombang maksimum tersebut yang digunakan untuk mengukur serapan dari sampel ekstrak etanol kulit buah alpukat *(Persea americana* Mill.*)*. Dapat dilihat pada tabel 3.

Tabel 3. Hasil pengukuran absorbansi larutan standar kuersetin pada panjang gelombang maksimum 435 nm

Konsentrasi (ppm)	Absorbansi (y)
6	0,278
8	0,378
10	0,442
12	0,555
14	0,628



Gambar 1. Kurva kalibrasi kuersetin pada panjang gelombang maksimum 435 nm

Dari pengukuran tersebut, dapat disimpulkan bahwa semakin tinggi konsentrasi yang digunakan maka semakin tinggi pula absorban yang di peroleh. Hasil baku kuersetin yang diperoleh diplotkan antara kadar dan absorbannya, sehingga diperoleh persamaan regresi linear yaitu $y = 0,0438x + 0,0177$ dengan nilai R^2 yang diperoleh sebesar 0,9944 dan nilai r adalah 0,997. Persamaan kurva kalibrasi kuersetin dapat digunakan sebagai pembanding untuk menentukan konsentrasi senyawa flavonoid total pada ekstrak sampel.

Pengujian analisis kuantitatif dengan spektrofotometri UV-Vis digunakan larutan blanko

sebagai kontrol yang berfungsi sebagai pemblank (mengkali nol-kan) senyawa yang tidak perlu dianalisis (Basset,1994).

Pada pengukuran senyawa flavonoid total, larutan sampel ditambahkan $AlCl_3$ yang dapat membentuk kompleks, sehingga terjadi pergeseran panjang gelombang ke arah *visible* (tampak) yang ditandai dengan larutan menghasilkan warna yang lebih kuning. Dan penambahan kalium asetat yang bertujuan untuk mempertahankan panjang gelombang pada daerah *visible* (tampak) (Chang *et al*, 2002). Perlakuan inkubasi selama 1 jam sebelum pengukuran dimaksudkan agar reaksi berjalan sempurna, sehingga intensitas warna yang dihasilkan lebih maksimal (Azizah dan Faramayuda 2014, h. 48). Sehingga dari hasil penelitian ini diperoleh kadar flavonoid total ekstrak etanol kulit buah alpukat *(Persea americana* Mill.*)* sebesar 4,0122 mgQE/g ekstrak yang dapat dilihat pada tabel 3.

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Tabel 4. Hasil penetapan kadar flavonoid total % (b/b) pada ekstrak etanol kulit buah alpukat *(Persea americana* Mill.*)*

Replikasi	Abs (y)	Kandungan flavonoid total awal (mg/L)	Kandungan total flavonoid (mgQE/g ekstrak)	Rata-rata kandungan flavonoid total (mgQE/g)
1	0,291	6,2397	4,1050	
2	0,276	5,8972	3,9054	4,0122
3	0,284	6,0799	4,0264	

Menurut penelitian yang telah dilakukan oleh kurniasari (2006) menyatakan bahwa sejumlah tanaman obat yang mengandung flavonoid telah dilaporkan memiliki aktivitas antioksidan, antibakteri, antivirus, antiradang, antialergi dan antikanker.

IV. KESIMPULAN

Berdasarkan hasil penelitian yang telah dilakukan dapat disimpulkan bahwa kadar flovonoid total dari ekstrak etanol kulit buah alpukat *(Persea americana* Mill.*)* yaitu 4,0122 mgQE/g ekstrak.

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Determination of total flavonoid content in avocado (*Persea americana* Mill.) using spectrofotometry method

Penetapan kadar flavonoid total alpukat (*Persea americana* Mill.) dengan metode spektrofotometri

Hani Asmorowati*, Novena Yety Lindawati

Jurusan Farmasi STIKES Nasional Surakarta, Sukoharjo, Jawa Tengah
Corresponding author. Email: haniasmorowati16@gmail.com

Abstract
Background: Flavonoids are antioxidants that can reduce insulin resistance, increase insulin sensitivity, and improve the functions of pancreatic beta cells. Sources of flavonoids can be found in avocados.
Objective: This study aimed to determine the total flavonoid content in two different type of avocado (*Persea americana* Mill.) using the Uv-Vis spectrophotometric method.
Method: In the Uv-Vis spectrophotometry, the wavelength was measured at 413.6 nm with AlCl3 reagent to form complex compounds.
Results: The average total flavonoid content in the ethanol extract of common avocados was 10.95% b/b with coefficient of variation of 0.33% and 10.31% b/b with 0.28% coefficient of variation in butter avocados.
Conclusion: The t-Test showed that the total flavonoid content in ordinary avocados and butter avocados differed significantly (p = 0.000 (<0.05)).
Keywords: flavonoids, avocados, spectrophotometry.

Intisari
Latar belakang: Flavonoid merupakan antioksidan yang dapat menurunkan resistensi insulin, meningkatkan sensitivitas insulin, serta memperbaiki fungsi sel-sel beta pankreas. Sumber flavonoid dapat ditemukan pada buah alpukat.
Tujuan: Penelitian ini bertujuan untuk menetapkan kadar flavonoid total dari dua varian buah alpukat (*Persea americana* Mill.) menggunakan metode spektrofotometri Uv-Vis.
Metode: Pada metode spektrofotometri Uv-Vis, diukur pada panjang gelombang 413,6 nm dengan reagen $AlCl_3$ sebagai pembentuk senyawa kompleks.
Hasil: Didapatkan kadar rata-rata flavonoid total pada ekstrak etanol buah alpukat biasa 10,95% b/b dengan koefisien variasi 0,33% dan pada alpukat mentega 10,31% b/b dengan koefisien variasi 0,28%.
Kesimpulan: Uji statistik *T-Test* menunjukkan bahwa kadar flavonoid total pada buah alpukat biasa dan alpukat mentega terdapat perbedaan yang signifikan (p = 0,000 (< 0,05)).
Kata kunci: flavonoid, alpukat, spektrofotometri

1. Pendahuluan

DM (Diabetes Mellitus) atau kencing manis merupakan salah satu jenis penyakit menahun, yang angka kejadiannya semakin meningkat dari tahun ke tahun. Data yang diperoleh dari lembaga kesehatan dunia atau *World Health Organization* (WHO) mencatat pada tahun 2000, penderita diabetes mellitus di Indonesia sebanyak 8,4 juta orang, dan diperkirakan akan

meningkat menjadi 21,3 juta penderita pada tahun 2030 mendatang. Penyakit ini dapat dicegah dengan cara memperbaiki pola gaya hidup. (Rudijanto, 2014).

Gaya hidup kembali ke alam (*back to nature*) menjadi cukup popular saat ini sehingga masyarakat kembali memanfaatkan berbagai bahan alam, termasuk pengobatan dengan tumbuhan obat. Tanaman berkhasiat obat mempunyai nilai lebih ekonomis dan efek samping lebih kecil dibandingkan dengan obat-obat sintetis. Salah satu tanaman yang berpotensi sebagai tanaman obat yaitu buah alpukat (*Persea americana* Mill.). Menurut Malangngi, *et al.* (2012), buah alpukat memiliki dua varietas, yaitu alpukat varietas merah bundar atau disebut alpukat biasa (*Persea americana* Mill.) dan varietas alpukat hijau panjang atau disebut alpukat mentega

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Menurut Fathonah, *et al*. (2014) flavonoid merupakan antioksidan yang dapat menurunkan resistensi insulin, meningkatkan sensitivitas insulin, serta memperbaiki fungsi sel-sel beta pankreas. Alpukat merupakan salah satu buah sumber flavonoid yang dapat digunakan sebagai buah pilihan bagi penderita diabetes mellitus. Tujuan dilakukan penelitian ini adalah untuk mengetahui kadar flavonoid total dalam ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dan alpukat mentega (*Persea americana* Mill.).

2. Metodologi penelitian

2.1. Alat dan bahan penelitian

Alat-alat yang digunakan dalam penelitian ini adalah pisau, kain hitam, blender, kertas saring, toples kaca, gelas beker (Pyrex), batang pengaduk, corong kaca (Pyrex), *rotatory evaporator* (RV 10 Basic V), neraca analitik (Ohaus EP214), kaca arloji, cawan penguap, gelas ukur (Pyrex), *chamber*, pipet tetes, pipet ukur (Iwaki), pipet volume (Iwaki), labu ukur (Iwaki), tabung reaksi (Iwaki), dan seperangkat alat spektrofotometri uv – vis (Shimadzu UV mini 1240).

Bahan-bahan yang digunakan dalam penelitian ini adalah buah alpukat biasa dan alpukat mentega, etanol 70%, HCl pekat, serbuk Mg, baku kuersetin (Sigma Aldrich), $AlCl_3$ 10% (Merck), asam asetat 5% (Merck), silika gel GF_{254} (Merck), etil asetat (Merck), kloroform (Merck), metanol (Merck), dan aquadest.

2.2. Pengambilan dan pengolahan sampel

Pengambilan sampel buah alpukat biasa (*Persea americana* Mill.) dan alpukat mentega (*Persea americana* Mill.) diperoleh dari kawasan pertanian di Tawangmangu RT 05 RW 02, Kabupaten Karanganyar, Jawa Tengah. Determinasi buah alpukat biasa dan alpukat mentega dilakukan dengan cara mencocokkan ciri-ciri morfologi yang ada pada buah alpukat biasa dan alpukat mentega di Laboratorium Biologi, Universitas Muhammadiyah Surakarta, Surakarta, Jawa Tengah. Daging buah alpukat biasa (*Persea americana* Mill.) dan alpukat mentega (*Persea*

americana Mill.) dilakukan pengubahan bentuk dengan cara dipotong-potong kecil dengan tebal 0,5 mm-1 mm, selanjutnya dikeringkan dengan cara diangin-anginkan selama beberapa hari pada udara terbuka dengan tidak terkena sinar matahari langsung, yaitu dengan ditutup kain hitam. Sampel kering kemudian diblender, sehingga didapatkan serbuk kering.

2.3. Pembuatan ekstrak

Masing-masing sebanyak 200 gram sampel kering buah alpukat biasa (*Persea americana* Mill.) dan buah alpukat mentega (*Persea americana* Mill.) dimasukkan ke dalam wadah maserasi. Ditambahkan dengan etanol 70% sebanyak 1,5 liter (1:7,5) sampai seluruh sampel terendam, kemudian ditutup dan dibiarkan selama 3 x 24 jam sambil sesekali diaduk. Maserat disaring dengan menggunakan kain flanel, dipisahkan antara ampas dan filtratnya. Ampas dimaserasi kembali dengan etanol 70% sebanyak 0.5 liter (1:2.5) selama 1 x 24 jam. Selanjutnya disaring menggunakan kain flanel untuk memperoleh filtrat. Semua filtrat disatukan dan dipekatkan dengan menggunakan *rotatory evaporator* sampai tidak ada lagi cairan yang menetes sehingga

dengan menggunakan *rotatory evaporator* sampai tidak ada lagi cairan yang menetes sehingga diperoleh ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dan buah alpukat mentega (*Persea americana* Mill.). Preparasi sampel dilakukan sebanyak 3 kali replikasi.

2.4. Uji kualitatif flavonoid

2.4.1. Uji flavonoid dengan pereaksi Wilstater

Masing-masing 100 mg ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dan alpukat mentega (*Persea americana* Mill.) ditambahkan beberapa tetes HCl pekat. Ditambahkan sedikit serbuk Mg. Hasil positif ditunjukkan dengan perubahan warna merah-orange (Yuda, *et al.*, 2013).

2.4.2. Uji flavonoid dengan pereaksi Smith-Metcalf

Masing-masing 100 mg ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dan alpukat mentega (*Persea americana* Mill.) ditambahkan beberapa tetes HCl pekat kemudian dipanaskan. Hasil positif jika memberikan warna putih (Yuda, *et al.*, 2013).

2.4.3. Kromatografi Lapis Tipis (KLT)

Fase diam yang digunakan adalah silika gel GF_{254} dan fase geraknya adalah kloroform : metanol (1:4) sebanyak 10 mL. Sebanyak 0,01 gram ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dan kuersetin standar, masing-masing dilarutkan dalam 0,5 mL etil asetat, kemudian ditotolkan pada jarak 1 cm dari tepi bawah lempeng KLT. Lempeng KLT dikeringkan dan di elusi. Bercak kromatogram (noda) yang dihasilkan diamati dengan penampak noda sinar ultraviolet 254 nm (Priyanto, *et al.*, 2014). Lempeng KLT disemprot dengan reagen semprot $AlCl_3$. Perlakuan yang sama juga dilakukan pada ekstrak etanol 70% buah alpukat mentega (*Persea americana* Mill.).

2.5. Uji kuantitatif kandungan flavonoid

2.5.1. Pembuatan larutan baku induk kuersetin 1000 ppm

Ditimbang sebanyak 100 mg baku standar kuersetin dan dilarutkan dengan etanol 70% sampai dengan 100 mL.

2.5.2. Pembuatan larutan baku kerja kuersetin 100 ppm

Larutan baku induk dipipet sebanyak 1 mL dan dicukupkan volumenya sampai 10 mL dengan etanol 70% sehingga diperoleh konsentrasi 100 ppm.

2.5.3. Pembuatan larutan blangko

Pipet 1 mL $AlCl_3$ 10% dan 8 mL asam asetat 5%, tambahkan etanol 70% sampai dengan 10 mL.

2.5.4. Penentuan operating time

Larutan baku kerja kuersetin 100 ppm diambil sebanyak 1 mL ditambahkan dengan 1 mL $AlCl_3$ 10% dan 8 mL asam asetat 5%. Larutan tersebut diukur absorbansinya pada panjang gelombang maksimum teoritis 415 nm (Ipandi, *et al.*, 2016) dengan interval waktu 2 menit sampai diperoleh absorbansi yang stabil. Diamati kurva hubungan antara absorbansi, waktu, dan tentukan *operating time*.

2.5.5. Penentuan panjang gelombang maksimum (λ maks) kuersetin

Larutan baku kerja kuersetin 100 ppm diambil sebanyak 1 mL ditambahkan dengan 1 mL $AlCl_3$ 10% dan 8 mL asam asetat 5%. Lakukan pembacaan dengan spektrofotometri Uv-Vis pada panjang gelombang 370-450 nm. Hasil panjang gelombang maksimum tersebut digunakan untuk mengukur serapan dari sampel ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dan alpukat mentega (*Persea americana* Mill.) (Sari & Ayuchecaria, 2017).

2.5.6. Pembuatan kurva baku kuersetin

2.5.6. Pembuatan kurva baku kuersetin

Larutan baku induk kuersetin 1000 ppm, kemudian dipipet sebanyak 0,2 mL; 0,3 mL; 0,4 mL; 0,5 mL; 0,6 mL dan ditambahkan etanol 70% sampai volumenya 5 mL sehingga diperoleh konsentrasi yaitu 40 ppm, 60 ppm, 80 ppm, 100 ppm, dan 120 ppm. Masing-masing konsentrasi dari seri baku kuersetin dipipet 1 mL, kemudian ditambahkan 1 mL $AlCl_3$ 10% dan 8 mL asam asetat 5%, didiamkan selama *operating time*. Absorbansi ditentukan menggunakan metode spektrofotometri Uv-Vis pada panjang gelombang maksimum yang diperoleh (Sari & Ayuchecaria, 2017).

2.5.7. Penetapan kadar flavonoid total ekstrak etanol 70% buah alpukat biasa (Persea americana Mill.) dan alpukat mentega (Persea americana Mill.)

Ditimbang 100 mg masing-masing ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dan alpukat mentega (*Persea americana* Mill.) dilarutkan dengan etanol 70% sampai volumenya 100 mL. Larutan tersebut masing-masing dipipet 1 mL kemudian ditambahkan 1 mL larutan $AlCl_3$ 10% dan 8 mL asam asetat 5%. Sampel didiamkan selama

operating time. Absorbansi ditentukan menggunakan metode spektrofotometri Uv-Vis pada panjang gelombang maksimum yang diperoleh (Sari & Ayuchecaria, 2017).

2.6. Analisis data penelitian

2.6.1. Perhitungan kadar

Kadar flavonoid dihitung dengan pengukuran ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dan alpukat mentega (*Persea americana* Mill.) kemudian dihitung dengan rumus:

$y = a + bx$

Dimana:

y = serapan (absorbansi)

a = intersep (titik potong kurva terhadap sumbu y)

b = kemiringan (slope) kurva linier

x = konsentrasi (ppm)

r = koefisien relasi

2.6.2. Perhitungan Koefisien Variasi (% KV)

Perhitungan % KV digunakan untuk mengetahui perbandingan antara simpangan kadar flavonoid total dengan rata-rata kadar sampel ekstrak etanol 70% buah alpukat biasa (Persea americana Mill.) dan alpukat mentega (Persea americana Mill.) yang dinyatakan dalam %. Nilai kefisien variasi dinyatakan baik apabila kurang dari 2% (Snyder, *et al.*, 2010). Koefisien variasi dirumuskan dengan:

$$\% \text{ KV} = \frac{\text{SD}}{\text{rata-rata kadar sampel}} \text{ X } 100\% \quad (1)$$

2.6.3. Uji Statistika

Uji *Independent T Test* digunakan untuk menguji perbedaan rata-rata antara dua kelompok Independen, sehingga uji ini digunakan untuk mengetahui adanya perbedaan kadar flavonoid total yang signifikan antara sampel ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dan alpukat mentega (*Persea americana* Mill.) (Riyanto, 2011).

3. Hasil dan pembahasan

Penelitian ini dilakukan untuk mengetahui kadar flavonoid total dalam ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dan alpukat mentega (*Persea americana* Mill.) dengan menggunakan metode spektrofotometri Uv-Vis. Metode spektrofotometri Uv-Vis dipilih karena metode yang sederhana, mudah, dan cepat dibandingkan dengan metode yang lain, selain itu dapat digunakan untuk analisis suatu zat berwarna maupun tidak berwarna dalam kadar kecil.

3.1. Preparasi sampel

Buah alpukat biasa dan alpukat mentega dilakukan determinasi dengan tujuan untuk menetapkan kebenaran sampel yang digunakan dalam penelitian. Hasil determinasi

menyatakan bahwa buah yang digunakan dalam proses penelitian yaitu buah alpukat biasa dengan nama spesies (*Persea americana* Mill.) termasuk ke dalam familia Lauraceae dan buah alpukat mentega dengan nama spesies (*Persea americana* Mill.) termasuk ke dalam familia Lauraceae.

Metode ekstraksi yang digunakan adalah maserasi. Proses ekstraksi dilakukan bertujuan untuk mengambil senyawa kimia yang terkandung dalam sampel. Pelarut yang digunakan pada maserasi adalah etanol 70% yang bersifat polar, sehingga dapat menarik secara maksimal senyawa flavonoid yang bersifat polar juga.

Maserasi dilakukan selama 3 x 24 jam dan selanjutnya ampas di maserasi kembali selama 1 x 24 jam dengan pelarut etanol 70% yang baru. Tujuan dilakukan maserasi kembali yaitu untuk memaksimalkan proses penyarian sehingga ekstrak yang didapat lebih maksimal. Pada proses maserasi cairan penyari akan menembus dinding sel dan masuk ke dalam rongga sel yang mengandung zat aktif. Pelarut yang telah menyari zat aktif akan ada pada kondisi terpekat dan akan didesak keluar karena adanya perbedaan konsentrasi antara larutan zat aktif di dalam sel dan dengan yang di luar sel (Depkes RI, 1989).

Seluruh filtrat yang diperoleh dipekatkan menggunakan *rotatory evaporator* dengan suhu maksimal 50°C dan dengan kecepatan 125 rpm. Maksimal suhu yang digunakan adalah 50°C bertujuan untuk menghindari terjadinya kerusakan zat aktif akibat pengaruh suhu tinggi. Tujuan dari pemekatan adalah untuk memisahkan pelarut etanol 70% dengan filtrat yang diperoleh sehingga didapatkan ekstrak kental.

Hasil organoleptis ekstrak buah alpukat biasa dan alpukat mentega yaitu berbentuk cairan kental, berbau khas, dan berwarna coklat tua. Hasil rendemen yang diperoleh pada ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dari tiga kali replikasi berturut-turut yaitu 22,25%, 20,60%, dan 20,45% serta untuk alpukat mentega (*Persea americana* Mill.) dari tiga kali replikasi berturut-turut yaitu 32,35%, 32,95%, dan 32,65%. Hasil rendemen yang diperoleh pada ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dan alpukat mentega (*Persea americana* Mill.) ditunjukkan pada tabel 1.

Tabel 1. Hasil rendemen sampel

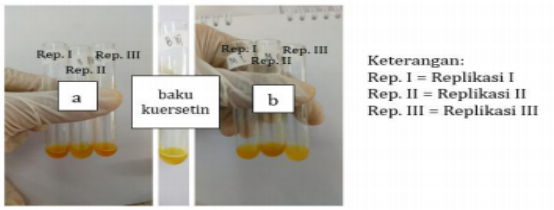
Sampel ekstrak etanol 70%	Hasil rendemen		
	Replikasi 1 (%)	Replikasi 2 (%)	Replikasi 3 (%)
Buah alpukat biasa	22,25	20,60	20,45
Buah alpukat mentega	32,35	32,95	32,65

3.2. Uji kualitatif

Uji kualitatif senyawa flavonoid dilakukan untuk mengetahui ada tidaknya senyawa flavonoid dalam sampel sebelum dilakukan uji kuantitatif. Uji kualitatif senyawa flavonoid meliputi pereaksi Wilstater, pereaksi Smith-Metcalfe, dan metode KLT.

3.2.1. Pereaksi Wilstater

Hasil identifikasi sampel ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dan alpukat mentega (*Persea americana* Mill.) dari tiga kali replikasi dengan penambahan HCl pekat dan serbuk Mg menunjukkan positif mengandung senyawa flavonoid dengan adanya perubahan warna dari coklat ekstrak menjadi orange. Hal ini sesuai dengan baku kuersetin sebagai pembanding yang memiliki perubahan warna yang serupa yaitu warna orange.

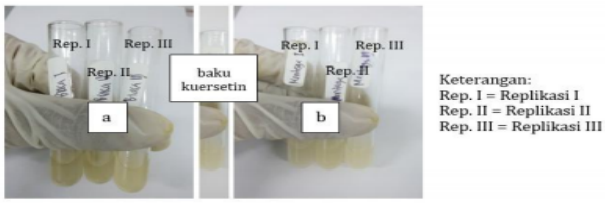


Gambar 1. Hasil uji flavonoid dengan pereaksi Wilstater buah alpukat biasa (a) dan buah alpukat mentega (b) positif mengandung flavonoid, ditunjukkan dengan warna orange yang serupa dengan baku kuersetin

Gambar 1 menunjukkan hasil uji flavonoid dengan pereaksi Wilstater. Penambahan HCl berfungsi untuk mendeteksi senyawa yang mengandung inti benzopiranon, sehingga setelah penambahan HCl akan menghasilkan garam benzopirilium yang disebut juga garam flavilium. Reduksi dengan Mg dan HCl menghasilkan senyawa kompleks yang berwarna orange pada flavonol.

3.2.2. Pereaksi Smith-Metcalfe

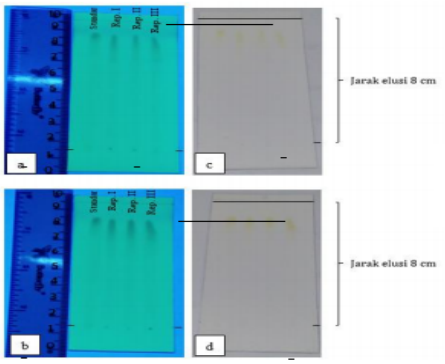
Hasil identifikasi sampel ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dan alpukat mentega (*Persea americana* Mill.) tiga kali replikasi dengan penambahan HCl pekat dan dipanaskan menunjukkan positif mengandung senyawa flavonoid dengan adanya perubahan warna dari coklat ekstrak menjadi putih kekuningan. Hal ini sesuai dengan kontrol baku kuersetin sebagai pembanding yang memiliki perubahan warna yang serupa yaitu warna putih kekuningan.



Gambar 2. Hasil uji flavonoid dengan pereaksi Smith-Metcalfe buah alpukat biasa (a) dan buah alpukat mentega (b) positif mengandung flavonoid, ditunjukkan dengan warna putih kekuningan yang serupa dengan baku kuersetin

Gambar 2 menunjukkan hasil uji flavonoid dengan pereaksi Smith-Metcalfe. Penambahan HCl pekat untuk menghidrolisis dan memutus ikatan glikosida. Pemanasan berfungsi untuk mempercepat reaksi hidrolisis yang terjadi, sehingga terjadi perubahan warna dari coklat ekstrak menjadi putih.

3.3. *KLT*



Gambar 3. Hasil KLT buah alpukat biasa (a) dan buah alpukat mentega (b) pada sinar UV 254 nm menunjukkan warna bercak kuning kecoklatan yang serupa dengan standar kuersetin. Hasil KLT buah alpukat biasa (a) dan buah alpukat mentega (b) setelah disemprot reagen $AlCl_3$ menunjukkan warna bercak kuning intensif yang serupa dengan standar kuersetin.

Gambar 3 menunjukkan hasil KLT positif mengandung flavonoid pada sampel ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dan alpukat mentega (*Persea americana*

Mill.) pada masing-masing replikasi. Hasil ditunjukkan dengan nilai HRf dan warna bercak yang sama yaitu warna kuning kecoklatan antara sampel dengan pembanding standar kuersetin. Penambahan reagen semprot $AlCl_3$ menunjukkan bercak warna kuning menjadi lebih intensif, hal ini terjadi karena adanya pembentukan senyawa kompleks. Pada fase gerak, jarak pengembangan yang ditempuh adalah 8 cm. Hasil KLT buah alpukat biasa dan alpukat mentega dapat dilihat pada tabel 2 dan 3.

Tabel 2. Hasil KLT buah alpukat biasa

Replikasi	hRf	Warna bercak pada sinar UV 254 nm	Penampak bercak $AlCl_3$
1	86	Kuning kecoklatan	Kuning intensif
2	86	Kuning kecoklatan	Kuning intensif
3	86	Kuning kecoklatan	Kuning intensif
Baku kuersetin	88	Kuning kecoklatan	Kuning intensif

Tabel 3. Hasil KLT buah alpukat mentega

Replikasi	hRf	Warna bercak pada sinar UV 254 nm	Penampak bercak $AlCl_3$
1	85	Kuning kecoklatan	Kuning intensif
2	85	Kuning kecoklatan	Kuning intensif
3	85	Kuning kecoklatan	Kuning intensif
Baku kuersetin	87	Kuning kecoklatan	Kuning intensif

Uji kualitatif ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dan ekstrak etanol 70% buah alpukat mentega (*Persea americana* Mill.) dengan pereaksi Wilstater, pereaksi Smith-Metcalfe, dan KLT diperoleh hasil positif mengandung flavonoid. Hasil uji kualitatif ditunjukkan pada tabel 4.

Tabel 4. Hasil uji kualitatif flavonoid

Uji Kualitatif Flavonoid	Standar baku kuersetin	Hasil	
		Ekstrak etanol 70% buah alpukat biasa	Ekstrak etanol 70% buah alpukat mentega
Pereaksi Wilstater	Warna orange	Warna orange (Positif)	Warna orange (Positif)
Pereaksi Smith-Metcalf	Warna putih	Warna putih (Positif)	Warna putih (Positif)
KLT	Warna bercak kuning kecoklatan	Warna bercak kuning kecoklatan (Positif)	Warna bercak kuning kecoklatan (Positif)

3.4. Uji kuantitatif

3.4.1. Penentuan operating time

Penentuan *operating time* bertujuan untuk mengetahui waktu pengukuran yang stabil yaitu ketika sampel bereaksi sempurna dan membentuk senyawa kompleks (Gandjar & Abdul, 2007). *Operating time* dilakukan dengan menggunakan larutan baku kuersetin 100 ppm dengan interval waktu 2 menit dan dilakukan selama 60 menit. Hasil penentuan *operating time* diperoleh pada menit ke 34.

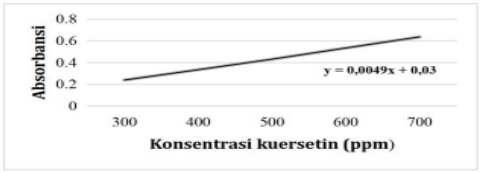
3.4.2. Penentuan panjang gelombang maksimum

Panjang gelombang maksimum adalah panjang gelombang yang dihasilkan suatu senyawa pada serapan maksimum (Gandjar & Abdul, 2007). Alasan dilakukan pengukuran pada panjang gelombang maksimum bertujuan untuk mengetahui panjang gelombang saat mencapai serapan maksimum, selain itu juga memiliki daya serap yang relatif konstan.

Penentuan panjang gelombang maksimum untuk kuersetin dengan cara membaca serapan larutan baku kerja kuersetin dengan konsentrasi 100 ppm pada panjang gelombang 370-450 nm. Hasil yang diperoleh dari penelitian ini yaitu 413,6 nm.

3.4.3. Penentuan kurva baku

Pembuatan kurva baku menggunakan larutan baku kuersetin dengan konsentrasi 40 ppm, 60 ppm, 80 ppm, 100 ppm, dan 120 ppm. Pemilihan konsentrasi didasarkan pada hukum Lambert-Beert yang menyatakan syarat serapan adalah 0,2-0,8 untuk menghindari terjadinya kesalahan fotometrik, sehingga kesalahan analisis masih dalam batas yang diterima yaitu 0,5-1 %. Pengukuran absorbansi dilakukan menggunakan panjang gelombang maksimum 413,6 dan *operating time* selama 34 menit.



Gambar 4. Kurva baku kuersetin yang diukur pada panjang gelombang 413,6 nm dengan *operating time* pada menit ke 34

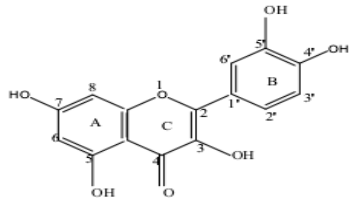
Gambar 4 menunjukkan bahwa konsentrasi berbanding lurus dengan nilai absorbansi

Gambar 4 menunjukkan bahwa konsentrasi berbanding lurus dengan nilai absorbansi, semakin besar konsentrasi larutan baku standar kuersetin maka semakin tinggi pula nilai absorbansi yang dihasilkan. Pada pengukuran absorbansi diperoleh persamaan regresi kuersetin y = 0,0049x + 0,03. Hasil nilai linearitas ditunjukkan dengan nilai koefisien korelasi (r)

sebesar 0,9999. Nilai (r) yang diperoleh mendekati angka 1 menunjukkan bahwa persamaan regresi tersebut adalah linier, sehingga dapat dikatakan bahwa absorbansi dan konsentrasi memiliki korelasi yang sangat kuat.

3.4.4. Penentuan kadar flavonoid total dalam sampel buah alpukat biasa (Persea americana Mill.) dan alpukat mentega (Persea americana Mill.)

Preparasi sampel dari masing-masing replikasi dilakukan sebanyak tiga kali, bertujuan untuk memperoleh data yang lebih akurat. Digunakan kuersetin sebagai larutan standar karena kuersetin merupakan flavonoid golongan flavonol yang mempunyai gugus keto pada C-4 dan memiliki gugus hidroksil pada atom C-3 atau C-5 yang bertetangga dari flavon dan flavonol. Struktur kuersetin dapat dilihat pada gambar 5.



Gambar 5. Struktur kuersetin (Azizah, *et al.*, 2014)

Kandungan flavonoid total ditentukan berdasarkan reaksi kolorimetri yaitu setelah sampel direaksikan dengan $AlCl_3$ dalam medium asam. Penambahan $AlCl_3$ dalam sampel dapat membentuk kompleks antara aluminium klorida dengan kuersetin sehingga terjadi pergeseran panjang gelombang ke arah visibel (tampak) dan ditandai dengan larutan menghasilkan warna yang lebih kuning. Fungsi penambahan asam asetat untuk mempertahankan panjang gelombang pada daerah visibel (tampak).

Tabel 5. Hasil penetapan kadar flavonoid buah alpukat biasa

Sampel / replikasi	Pengulangan tiap replikasi	Kadar (%)	Rata-rata kadar (%)	SD	KV (%)
Buah alpukat biasa 1	1	10,88	10,94	0,0360	0,33%
	2	10,96			
	3	10,98			
Buah alpukat biasa 2	1	10,92	10,92		
	2	10,90			
	3	10,94			
Buah alpukat biasa 3	1	10,96	10,99		
	2	11,00			
	3	11,00			

Berdasarkan data pada tabel 5 dapat dilihat bahwa sampel ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) memiliki kadar rata-rata flavonoid total yaitu 10,95% dengan koefisien variasi sebesar 0,33%.

Tabel 6. Hasil penetapan kadar flavonoid buah alpukat mentega

Sampel / replikasi	Pengulangan tiap replikasi	Kadar (%)	Rata-rata kadar (%)	SD	KV (%)
Buah alpukat mentega 1	1	10,22	10,29		
	2	10,29			
	3	10,35			
Buah alpukat mentega 2	1	10,33	10,34	0,0289	0,28%
	2	10,33			
	3	10,35			
Buah alpukat mentega 3	1	10,26	10,29		
	2	10,31			
	3	10,31			

Pada tabel 6 dapat dilihat bahwa ekstrak etanol 70% buah alpukat mentega (*Persea americana* Mill.) memiliki kadar rata-rata flavonoid total yaitu 10,31% dengan koefisien variasi sebesar 0,28%. Hal ini menunjukkan kadar rata-rata flavonoid total pada sampel ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) relatif lebih tinggi dibandingkan dengan alpukat biasa (*Persea americana* Mill.). Nilai koefisien variasi pada sampel buah alpukat biasa dan alpukat mentega memenuhi persyaratan koefisien variasi karena kurang dari 2%. Hal tersebut menunjukkan bahwa data pada penetapan kadar flavonoid total dalam buah alpukat biasa dan alpukat mentega diperoleh dengan tingkat ketelitian kerja yang baik.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
KADAR FLAVONOID TOTAL	Equal variances assumed	.031	.862	31.885	16	.000	.64333	.02018	.60056	.68611
	Equal variances not assumed			31.885	15.998	.000	.64333	.02018	.60056	.68611

Gambar 6. Hasil uji statistik *Independent Samples T-Test*

Gambar 6 menunjukkan hasil uji statistika *Independent Samples T-Test*. Uji statistika menggunakan uji *Independent Samples T-Test* dilakukan untuk mengetahui perbandingan kedua kadar dengan tingkat kepercayaan 95%. Hasil uji *Independent Samples T-Test* menunjukkan di

kolom *sig.* (*2 tailed*) yaitu sebesar p = 0,000 (< 0,05) yang artinya ada perbedaan signifikan rata-rata kadar flavonoid total pada sampel ekstrak etanol 70% buah alpukat biasa (*Persea americana* Mill.) dengan alpukat mentega (*Persea americana* Mill.).

4. Kesimpulan

Berdasarkan hasil penelitian menunjukkan bahwa buah alpukat biasa (*Persea americana* Mill.) memiliki kadar rata-rata flavonoid total sebesar 10,95% dengan koefisien variasi sebesar 0,33% relatif lebih tinggi dibandingkan dengan alpukat mentega (*Persea americana* Mill.) sebesar 10,31% dengan koefisien variasi sebesar 0,28%.

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Daftar pustaka

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DETERMINATION OF TOTAL FLAVONOID LEVELS ON ALPUKAT FRUIT SKIN (PERSEA AMERICANA MILL.)

Firlia dan Sri Hastuti

Jurusan Pendidikan MIPA
Fakultas Keguruan dan Ilmu Pendidikan Universitas Tadulako Palu

Abstract

Avocados (Persea americana Mill) are fruits that originally from Mexico and Central America. It has the characteristics of green flesh on the bottom of the skin and yellowing toward the seeds with a texture that is rather soft when it is ripe. Skin color varies, some are green because of chlorophyll or black content due to anthocyanin pigments. This study aimed to determine the total levels of flavonoids in the skin of avocados that were green and black. Determination of total flavonoid levels used a UV- Vis spectrophotometer. The results showed that an analysis of water content was 5.306% for green avocado skin and 7.327% for black avocado skin. The analysis of total flavonoid levels at a wavelength of 437 nm obtained respectively, the average yield was 54,950 mg/100g for green avocado skin and 29,519 mg/100g for black avocado skin. The results of this study are expected to be able to attract the interest of the community to cultivate green and black avocado plants. especially in the area of Central Sulawesi.

Keywords: *Black and green avocado skin, flavonoids, UV-Vis spectrophotometer.*

PENDAHULUAN

Indonesia terkenal dengan keanekaragaman jenis buah dan negara yang memiliki kekayaan alam dengan berbagai jenis tanaman yang berkhasiat sebagai obat tradisional. Salah satunya adalah buah alpukat (Persea americana Mill.). Alpukat (Persea americana Mill.) merupakan tanaman yang dapat tumbuh subur di daerah tropis seperti Indonesia (Feliana, dkk. 2018). Buah- buahan yang berwarna cerah umumnya memiliki aktivitas antioksidan yang baik bagi tubuh diantaranya buah alpukat (Rahmi, 2017). Kulit alpukat merupakan limbah yang memiliki banyak khasiat yang dapat bermanfaatkan bagi manusia (Fauziah, 2016). Alpukat tidak mengandung kolestrol atau sodium dan rendah lemak jenuh (Antasionasti, 2016) .

Kulit alpukat mengandung senyawa flavonoid yang dapat digunakan untuk melindungi kulit terhadap sinar UV atau mampu mengurangi kerusakan kulit (Mokodompit et al., 2013). Buah alpukat yang masak memiliki kandungan metabolit sekunder (flavonoid, tanin dan antosianin) yang lebih besar pada biji dan kulit buahnya dibandingkan pada buah alpukat yang masih muda (Yachya & Sulistyowati, 2015). Demikian juga kulit ekstrak alpukat mempunyai beberapa kandungan karotein, fenolik total, dan flavonoid yang lebih tinggi dari pada daging buahnya (Vinha et al., 2013).

Kulit buah alpukat memiliki aktivitas antibakteri (Wulandari et al., 2019). Diketahui sebagai antibakteri karena kandungan senyawa antibakteri seperti saponin, alkaloid, dan flavonoid pada buah dan daunnya (Ernawati, 2015).

Salah satu kandungan senyawa yang terdapat di dalam buah-buah tersebut adalah senyawa flavonoid (Febrianti & Sari, 2016). Flavonoid merupakan suatu bahan aktif yang dapat berperan sebagai antioksidan (Wahyuni, 2019). Antioksidan dapat menjadi strategi ampuh untuk mengurangi kerusakan akibat radikal bebas (Dikici et al., 2017).Flavonoid juga dapat menangkap radikal bebas yang dapat merusak sel tubuh (Shinta, 2018). Pada kulit buah alpukat kandungan kimianya yang lebih berperan yaitu flavonoid karena merupakan salah satu senyawa golongan fenol alam yang terbesar yang terdapat dalam semua tumbuhan hijau (Jayustin & Fratama, 2019). Kulit buah alpukat juga dapat digunakan untuk sintesis nanopartikel perak (Agnes Rantesalu, 2019).

Flavonoid adalah pigmen tumbuhan yang memberikan manfaat kesehatan bagi konsumen manusia dan hewan (Paauw et al., 2019). Analisis tersebut digunakan untuk melakukan uji secara kuantitatif untuk menentukan jumlah flavonoid yang terdapat dalam ekstrak yang dilakukan dengan mengukur nilai absorbansinya. Absorbansi dan kadar flavonoid memiliki hubungan yang linear, yaitu semakin tinggi absorbansi yang terukur maka kadar flavonoid yang terkandung didalam suatu tanaman juga semakin tinggi (Aminah et al., 2017).

Berdasarkan uraian diatas untuk meningkatkan pemanfaatan kulit buah alpukat sebagai sumber obat tradisional, maka peneliti tertarik untuk meneliti kadar flavonoid total yang terkandung pada ekstrak kulit buah alpukat hijau dan hitam dengan menggunakan metode

spektrofotometri UV-Vis, untuk membandingkan kulit buah alpukat manakah yang memiliki kadar flavonoid lebih besar yang berpotensi sebagai obat.

METODE PENELITIAN

Alat dan bahan

Alat-alat yang digunakan pada penelitian ini yaitu pipet tetes, tabung reaksi, neraca digital, spatula, Erlenmeyer 100 mL, mikro pipet, gelas kimia 100 mL, labu ukur 50 mL, shaker, corong, kertas saring, gelas ukur 100 mL, rak tabung reaksi, oven, cawan, penjepit, wadah, kuvet, spektrofotometer UV-Vis, sendok, desikator,

b. Pembuatan larutan standar kuersetin

Ditimbang 10 mg standar baku kuersetin kemudian dimasukkan ke dalam labu ukur 50 mL, ditambahkan etanol 95 % sampai tanda batas (larutan induk 1000 mg/L). Kemudian dibuat serangkaian larutan standar 20, 40, 60, 80, dan 100 mg/L. Pipet masing-masing larutan standar 1 mL, lalu tambahkan 1,5 mL etanol 95%, 0,5 mL aluminium klorida (AlCl3) 10%, 0,5 mL kalium asetat 1 M dan tambahkan akuades 2,8 mL. setelah itu, diinkubasi selama 30 menit pada suhu kamar. Absorbansi ditentukan menggunakan metode spektrofotometer UV-Vis pada panjang gelombang 437 nm, kemudian dibuat kurva kalibrasinya.

spektrofotometri UV-Vis, untuk membandingkan kulit buah alpukat manakah yang memiliki kadar flavonoid lebih besar yang berpotensi sebagai obat.

METODE PENELITIAN

Alat dan bahan

Alat-alat yang digunakan pada penelitian ini yaitu pipet tetes, tabung reaksi, neraca digital, spatula, Erlenmeyer 100 mL, mikro pipet, gelas kimia 100 mL, labu ukur 50 mL, shaker, corong, kertas saring, gelas ukur 100 mL, rak tabung reaksi, oven, cawan, penjepit, wadah, kuvet, spektrofotometer UV-Vis, sendok, desikator, blender dan gunting.

Bahan yang digunakan pada penelitian ini yaitu sampel ekstrak kulit buah alpukat hijau dan hitam (*Persea americana Mill*), etanol 95 % , etanol 96 %, aluminium foil, tissue, aluminium klorida (AlCl3) 10%, kalium asetat, aquades, standar kuarcetin konsesntrasi 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm. Alat yang digunakan pada penelitian ini yaitu palu-palu, lumpeng dan alu, cawan porselin, ayakan 70 mesh, oven, neraca analitik, desikator, serta seperangkat alat XRF. Bahan yang digunakan pada penelitian ini yaitu batuan dari pertambangan emas rakyat Poboya dan aquades.

Penyiapan sampel

Buah alpukat yang berwarna hijau dan hitam pada tahap ini dikupas dan diambil kulitnya lalu di cuci sampai bersih menggunakan air mengalir. Setelah itu kulit alpukat hijau dan hitam dipotong kecil-kecil, selanjutnya dikeringkan dengan cara diangin-anginkan selama seminggu pada suhu ruang dengan tidak terkena sinar matahari langsung. Setelah kering sampel ditimbang dan dicatat berat keringnya kemudian diserbukkan setelah itu ditimbang kembali berat sampel serbuk yang di peroleh (Rizki, dkk. 2016).

Penentuan kadar flavonoid total

Penetapan kadar flavonoid total dengan metode kolorimetri yang mengacu pada prosedur (Chang et al., 2002) dan (Ahmad et al., 2014) dengan beberapa modifikasi dengan kuersetin (QE) sebagai standar.

a. Penentuan panjang gelombang maksimum (λmaks) kuarsetin

Penentuan panjang gelombang maksimum kuarsetin dengan running larutan kuarsetin pada range panjang gelombang 400-500 nm. Hasil running menunjukkan panjang gelombang maksimum standar baku kuarsetin berada pada panjang gelombang 437 nm. Panjang gelombang maksimum tersebut yang digunakan untuk mengukur serapan dari sampel ekstrak kulit buah alpukat (persea americana Mill.).

b. Pembuatan larutan standar kuersetin

Ditimbang 10 mg standar baku kuersetin kemudian dimasukkan ke dalam labu ukur 50 mL, ditambahkan etanol 95 % sampai tanda batas (larutan induk 1000 mg/L). Kemudian dibuat serangkaian larutan standar 20, 40, 60, 80, dan 100 mg/L. Pipet masing-masing larutan standar 1 mL, lalu tambahkan 1,5 mL etanol 95%, 0,5 mL aluminium klorida (AlCl3) 10%, 0,5 mL kalium asetat 1 M dan tambahkan akuades 2,8 mL. setelah itu, diinkubasi selama 30 menit pada suhu kamar. Absorbansi ditentukan menggunakan metode spektrofotometer UV-Vis pada panjang gelombang 437 nm, kemudian dibuat kurva kalibrasinya.

c. Penentuan kadar flavonoid total

Menimbang sampel kulit buah alpukat hijau dan hitam sebanyak 1 gram menggunakan neraca digital. Kemuadian memasukan sampel kedalam erlenmeyer 100 mL, lalu menambahkan etanol 96% sebanyak 50 mL, sampai seluruh sampel terendam, kemudian ditutup dan dibiarkan selama 24 jam. Maserat disaring dengan menggunakan kertas saring. Filtrat diperoleh melalui penyaringan dengan corong, kemudian ampas dimaserasi kembali dengan etanol 96% 50 mL, sehingga filtrat hampir tidak berwarna. Semua filtrat disatukan dan dipekatkan dengan menggunakan rotavapor sampai tidak ada lagi cairan yang menetes sehingga diperoleh ekstrak etanol kulit buah alpukat (Persea americana Mill.). Ekstrak kental kulit buah alpukat (Persea americana Mill.) yang didapatkan digunakan untuk dianalisis lebih lanjut.

Selanjutnya menambahkan etanol 95 % sebanyak 1,5 mL. Selanjutnya menambahkan aluminium klorida (AlCl3) sebanyak 1 ml pada tabung, lalu menambahkan kalium asetat 0,5 mL pada tabung. Kemudian menambahkan aquades sebanyak 2,8 ml pada tabung. Kemudian mendiamkan selama 30 menit, lalu menyaring kembali larutan untuk memisahkan filtrat dan residu.kemudian memasukan filtrat kedalam kuvet kemudian diukur nilai serapannya menggunakan alat spektrofotometri UV-Vis dengan panjang gelombang 437 nm penentuan total flavonoid dengan metode kolorimetri.

ANALISA DATA

Menentukan Kadar Air

Teknik analisa data pada penelitian ini terlebih dahulu dilakukan dengan menentukan kadar air :

$$\%\ \text{Kadar air} = \frac{((BS - (rata - rata\ pengovenan - BS))}{BS} \text{x } 100\%$$

Menentukan Kadar Flavonoid

Data larutan standar ini digunakan untuk membuat persamaan regresi yaitu persamaan yang digunakan untuk menghitung kadar flavonoid :

$$y = ax + b$$

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$$F = \frac{C.\ V}{m} x100$$

Dimana F = Kadar flavonoid total (mg/100g), bahan x adalah konsentrasi sampel (mg/L), V adalah volume (L) dan m adalah massa sampel (g).

HASIL DAN PEMBAHASAN

Tumbuhan alpukat (Persea americana Mill.) yang digunakan dalam penelitian ini yaitu hanya pada bagian kulit buah alpukat. Kulit buah alpukat dapat digunakan sebagai bahan aktif tabir surya yaitu untuk melindungi kulit terhadap sinar UV atau mampu mengurangi kerusakan kulit, karena mengandung senyawa flavonoid (Mokodompit et al., 2012)

komponen zat yang terlarut kedalam pelarut sehingga terjadi perpindahan pada lapisan antar muka dan berdifusi masuk kedalam pelarut (Harbone, J.B. 1987). Semakin lama waktu ekstraksi, maka kadar total flavonoid semakin meningkat. Hal ini terjadi karena semakin lama proses ekstraksi, maka kontak antara pelarut dengan zat terlarut semakin lama dan pada waktu tertentu akan mencapai titik kesetimbangan (Yue. 2015). Menurut (Adawiah, 2015) bahwa flavonoid larut pada pelarut aquades, etanol dan methanol.Pelarut yang digunakan pada penelitian ini adalah etanol 96 % sebagai pelarut polar. Dalam hal penyarian etanol digunakan karena memiliki kelebihan dibandingkan dengan air dan metanol. Senyawa kimia yang mampu disari dengan etanol lebih banyak dari pada penyari metanol dan air. Hasil ekstraksi kulit buah alpukat

HASIL DAN PEMBAHASAN

Tumbuhan alpukat (Persea americana Mill.) yang digunakan dalam penelitian ini yaitu hanya pada bagian kulit buah alpukat. Kulit buah alpukat dapat digunakan sebagai bahan aktif tabir surya yaitu untuk melindungi kulit terhadap sinar UV atau mampu mengurangi kerusakan kulit, karena mengandung senyawa flavonoid (Mokodompit et al., 2013).

Flavonoid hampir terdapat pada semua bagian tumbuhan termasuk buah, akar, dan kulit luar batang. Flavonoid merupakan senyawa alam yang berpotensi sebagai antioksidan dan dapat menangkat radikal bebas yang berperan pada timbulnya penyakit degeneratif melalui mekanisme perusakan sistem imunitas tubuh, oksidasi lipid dan protein (Rais, 2015).

Pada penelitian ini buah alpukat yang digunakan diperoleh di sekitaran Kelurahan Tondo, Sulawesi Tengah. Kulit buah alpukat hijau dan hitam (Persea americana Mill.) dilakukan pengubahan bentuk dengan cara dipotong-potong kecil dan diangin-anginkan tujuannya untuk mempercepat proses pengeringan sehingga mengurangi kadar air yang terdapat pada sampel dan dapat mencegah pembusukan oleh bakteri.

Setelah kering sampel kulit buah alpukat hijau dan hitam berwarna hijau kecoklatan dan hitam kecoklatan, kemudian sampel dihaluskan sampai menjadi serbuk,.kemudian hasil pada sampel kulit buah alpukat ditimbang, untuk kulit buah alpukat hijau sebesar 6 gram berwarna coklat muda dan untuk kulit buah alpukat hitam sebesar 6 gram berwarna coklat tua. Kemudian selanjutnya dilakukan penentuan pada kadar air dari sampel tersebut.

Analisis kadar flavonoid pada Kulit Buah Alpukat Hijau dan Hitam

Penentuan kadar flavonoid total di awali dengan proses ekstraksi. Proses ekstraksi dilakukan terkandung dalam sampel. Prinsip ekstraksi didasarkan pada perpindahan masa dengan zat terlarut semakin lama dan pada waktu tertentu akan mencapai titik kesetimbangan (Yue. 2015). Menurut (Adawiah, 2015) bahwa flavonoid larut pada pelarut aquades, etanol dan methanol.Pelarut yang digunakan pada penelitian ini adalah etanol 96 % sebagai pelarut polar. Dalam hal penyarian etanol digunakan karena memiliki kelebihan dibandingkan dengan air dan metanol. Senyawa kimia yang mampu disari dengan etanol lebih banyak dari pada penyari metanol dan air. Hasil ekstraksi kulit buah alpukat hijau berwarna hijau bening dan buah alpukat hitam berwarna coklat bening.

Pada penelitian ini untuk menentukan kadar flavonoid total pada sampel kulit buah alpukat hijau dan hitam, dilakukan dengan pembuatan standar kuarsetin dengan deret konsetrasi larutan standar 20, 40, 60, 80 dan 100 ppm.

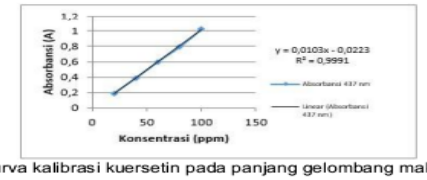
Digunakan deret konsentrasi karena metode yang dipakai dalam menentukan kadar adalah metode yang menggunakan persamaan

kurva baku, untuk membuat kurva baku terlebih dahulu dibuat beberapa deret konsentrasi untuk mendapatkan persamaan linear yang dapat digunakan untuk menghitung persen kadar. Digunakan kuarsetin sebagai larutan standar karena kuarsetin merupakan flavonoid golongan flavonol yang mempunyai gugus keto pada C-4 dan memiliki gugus hidroksi pada atom C-3 atau C-5 yang bertetangga dari flavon dan flavonol (Azizah et al., 2014). Pengukuran serapan panjang gelombang maksimum dilakukan running dari panjang gelombang 400-500 nm. Hasil running menunjukan panjang gelombang maksimum standar baku kuarsetin berada pada panjang gelombang 437 nm. Panjang gelombang maksimum tersebut yang digunakan untuk mengukur serapan dari sampel ekstrak kulit alpukat hijau dan hitam (*Persea americana Mill.*).

Kurva larutan standar kuersetin dibuat dengan mengasumsikan bahwa sumbu x adalah konsentrasi larutan kuersetin dan sumbu y merupakan absorbansi larutan kuersetin. Kurva ini akan digunakan untuk menentukan kadar flavonoid total sampel.

Tabel 1. Hasil pengukuran absorbansi larutan standar kuersetin pada panjang gelombang maksimum 437 nm.

Konsentrasi (ppm)	Absorbansi (y)
20	0,192
40	0,385
60	0,598
80	0,792
100	1,022



Gambar 1. Kurva kalibrasi kuersetin pada panjang gelombang maksimum 435 nm

Dari pengukuran tersebut, dapat disimpulkan bahwa semakin tinggi konsetrasi yang digunakan

(Chang et al., 2002). Dan untuk mendeteksi adanya gugus 7 hidroksi.

Dari pengukuran tersebut, dapat disimpulkan bahwa semakin tinggi konsetrasi yang digunakan maka semakin tinggi pula absorban yang di peroleh. Hasil standar kuarsetin yang diperoleh dari kurva standar kuarsetin, sehingga diperoleh persamaan regresi linear yaitu y = 0,0103x - 0,0223 dengan nilai R^2 yang diperoleh sebesar = 0,9991 dan nilai r mendekati 1. Persamaan kurva kalibrasi kuarsetin dapat digunakan sebagai pembanding untuk menentukan konsentrasi senyawa flavonoid total pada ekstrak sampel kulit alpukat hijau dan hitam.

Pada pengukuran senyawa flavonoid total AlCl3 yang dapat membentuk senyawa kompleks, sehingga terjadi pergeseran panjang gelombang kearah visible (tampak). Dan penambahan kalium asetat yang bertujuan untuk mempertahankan panjang gelombang pada daerah visible (tampak) (Chang et al., 2002). Dan untuk mendeteksi adanya gugus 7 hidroksi.

Setelah pendiaman selama 30 menit larutan berwarna putih keruh dan kuning keruh. Perlakuan inkubasi selama 30 menit sebelum pengukuran dimaksudkan agar reaksi berjalan sempurna, sehingga intensitas warna yang dihasilkan lebih maksimal (Azizah et al., 2014). Kemudian hasil penyaringan kedua sampel larutan berwarna bening.

Pengujian analisis kadar flavonoid dengan menggunakan spektrofotometri UV-Vis digunakan larutan blanko sebagai kontrol yang berfungsi sebagai pemblank (mengkali nol-kan) senyawa yang tidak perlu dianalisis. Sehingga hasil penelitian didapatkan rata-rata kulit buah alpukat hijau sebesar 54,950 mg/100g dan untuk kulit buah alpukat hitam sebesar 29,519 mg/100g.

Tabel 2. Data kadar flavonoid total

Sampel	Perlakuan	Absorbansi	Konsentrasi Flavonoid (mg/L)	Kadar Flavonoid (mg/100g)	Rata-rata Kadar Flavonoid (mg/100g)
Alpukat kulit hitam	1	0,023	4,500	22,388	29,519
	2	0,035	5,700	28,358	
	3	0,054	7,600	37,811	
Alpukat kulit hijau	1	0,078	10,000	49,505	54,950
	2	0,091	11,300	55,941	
	3	0,098	12,000	59,406	

KESIMPULAN

Berdasarkan hasil penelitian yang telah dilakukan dapat disimpulkan bahwa kadar flavonoid total dari ekstrak kulit alpukat hijau dan hitam (Persea americana Mill.) yaitu hijau sebesar 54,950 mg/100g dan hitam sebesar 29,519 mg/100g.

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Physicochemical Parameters, Phytochemical Composition and Antioxidant Activity of the Algarvian Avocado (*Persea americana* Mill.)

Ana F. Vinha[1,2], Joana Moreira[1] & Sérgio V. P. Barreira[1]

[1] FCS/UFP-Faculdade de Ciências da Saúde, Universidade Fernando Pessoa (FCS-UFP), Rua Carlos da Maia, Porto, Portugal

[2] REQUIMTE/Departamento de Ciências Químicas, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia da Universidade do Porto, Rua de Jorge Viterbo Ferreira, Porto, Portugal

Correspondence: Ana F. Vinha, Faculdade de Ciências da Saúde, Universidade Fernando Pessoa (FCS-UFP), Rua Carlos da Maia, Porto, Portugal. Tel: 352-225-074-630. E-mail: acvinha@ufp.edu.pt

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Abstract

The physical, chemical and nutritional properties of *Persea americana* fruits variety 'Hass' produced in the Algarve region were studied. Edible and non-edible parts of the fruits (pulp, seeds and peel) were compared considering their possible contribution to improve the sustainability of the food and pharmaceutical industries. The nutritional contents evaluated were moisture, ash, proteins, fat, total soluble solids and acidity. It were also evaluated the contents of bioactive compounds (phenolics, flavonoids, carotenoids, ascorbic acid and vitamin E) and their influence in the antioxidant activity exhibited by the fruit material. The results of the analysis demonstrated that the Algarvian avocado has physical and chemical characteristics comparable or superior to avocados from other growing regions around the world namely, Mexico and California. With regard to the contents of bioactive compounds, the pulp of the Algarvian avocado proved to be rich in carotenoids (0.815±0.201 mg/100g), phenolic compounds (410.2±69.0 mg/100g) and flavonoids (21.9±1.0 mg/100g). The skin was superior to the pulp in the contents of all these compounds with 2.585±0.117 mg/100g of carotenoids, 679.0±117.0 mg/100g of total phenolics and 44.3±3.1 mg/100g of flavonoids. The seed, in turn, was the part of the fruit with the highest total phenolic content (704.0±130.0 mg/100g) and flavonoids (47.97±2.69 mg/100g). Regarding the concentration of vitamins C and E, the highest values were found in the pulp (5.36±1.77 mg/100g of vitamin E) and skin (4.1±2.7 mg/100g of vitamin C). The extracts obtained from the seeds demonstrated higher *in vitro* DPPH• assay antioxidant activity (43%) than those obtained from the skin (35%) and the fruit pulp (23%). The contents of carotenoids, phenolic compounds and flavonoids found in the non-edible parts of the Algarvian avocado demonstrated that these byproducts could be an interesting inexpensive raw material for the food and cosmetic industries.

Keywords: *Persea americana* Mill., Algarvian avocado, food byproducts, bioactive compounds, antioxidant activity, edaphoclimatic conditions

1. Introduction

The *Persea americana* Mill. tree belongs to the family Lauraceae, genus *Persea* and is a plant native of Central America. Apart from its use as food the avocado is traditionally utilized for various medicinal purposes including as hypotensive, hypoglycemic and anti-viral, and is applied for the treatment of ulcers and cardiovascular diseases (Anita et al., 2005; Nayak et al., 2008; Raharjo et al., 2008; Anaka et al., 2009; Kosińska et al., 2012). To the avocados are equally attributed analgesic and anti-inflammatory properties (Adeyemi et al., 2002) and the avocado pulp is also used in various dermatological formulations namely, emulsions for the treatment of dry skin, protective agents against ultraviolet radiation, and anti-aging agents (Korać & Khambholja, 2011). Given the variety of uses that are assigned to ethnobotanical species *Persea americana* several studies have been conducted in order to unveil their biological activity (Gomez-Flores et al., 2008; Yasir et al., 2010; Pahua-Ramos et al., 2012). For example the characterization of phenolic components and antioxidant activity of hydroethanolic extracts of the avocado skin and seed revealed a predominance of compounds belonging to the group of

flavonoids, proanthocyanidins, and hydrocinnamic acids (Kosińska et al., 2012). Phenolics and flavonoids are bioactive compounds that have been related with a decrement of different deteriorative processes in the human body owing to their ability to reduce the formation and to scavange free radicals (Hidalgo et al., 2010). Rodríguez-Carpena and coworkers (2011) ascribed the high antioxidant activity exhibited by avocado extracts in various *in vitro* assays to these phenolic compounds. Chia and Dykes (2010) studied the essential oils of avocado and were able to demonstrate the antimicrobial activity of the skin and seeds of three different varieties of avocado ('Hass', 'Fuerte' and 'Shepard'). Other studies revealed that the avocado contains other classes of bioactive compounds with antioxidant properties and that are equally beneficial to Human metabolism, such as mineral constituents (phosphorus, magnesium and potassium), hydro and liposoluble vitamins (vitamin E, B, C and β-carotene, or provitamin A) (Honarbakhsh & Schachter, 2009; USDA, 2011). Given all the above, prominence has been given in certain countries, to public information about the avocado and its health promoting properties. An independent Australian organization, "The Heart Foundation" certified the fruit as healthy food for the heart and this certification with its appropriate logo is already used in advertising. The Californian Avocado Commission, has also driven efforts to publicize the fruit as health promoter, including conjoint publications with the American Dietetic Association, American Heart Association, and more recently, some press releases. For all the reasons above, the avocado is gaining worldwide recognition as healthy food and,

and β-carotene, or provitamin A) (Honarbakhsh & Schachter, 2009; USDA, 2011). Given all the above, prominence has been given in certain countries, to public information about the avocado and its health promoting properties. An independent Australian organization, "The Heart Foundation" certified the fruit as healthy food for the heart and this certification with its appropriate logo is already used in advertising. The Californian Avocado Commission, has also driven efforts to publicize the fruit as health promoter, including conjoint publications with the American Dietetic Association, American Heart Association, and more recently, some press releases. For all the reasons above, the avocado is gaining worldwide recognition as healthy food and, consequently, a significant economic value. Hence, quite naturally, the avocado culture has attracted the interest of European farmers and, currently, it is already possible to find avocado orchards spread across Spain, Italy, Greece and Portugal. Regarding cultivars produced, 'Hass' and 'Fuerte' dominate the international market (Rodríguez-Carpena et al., 2011). In Portugal these fruits are being cultivated in the south (Algarve), where the soil and climatic conditions are more favorable. The cultivated area at present does not exceed 750 acres but it is rapidly expanding, as more and more farmers recognize the potential of this crop (Freire, 2012). The avocado tree is one of the most productive plants per unit of cultivated area. The Algarve region has a temperate Mediterranean climate, characterized by mild short winters and long, hot and dry summers. The soils of this region are mostly litholic not humic of sandstone, stoneware of Silves or similar. Given that the edaphoclimatic conditions play a fundamental role in plant metabolism and by this route in the chemical makeup of fruits, one of the objectives of this study was to evaluate the chemical and antioxidant composition of the Algarvian 'Hass' avocado and compare their content of phytochemicals with those of the same variety of fruit produced elsewhere. This is pioneering study, since, to the best of our knowledge, this is the first scientific characterization of the Portuguese avocado fruit. The non-edible parts of the fruit (skin and seed) were also studied in order to assess their potential use as cheap source of bioactive compounds for the food, pharmaceutical and dermocosmetic industries. The exploitation of non-edible parts of the fruits is an emerging trend which may prove to be very profitable in the near future. Firstly because it entails an important reduction in the production of waste, secondly, because the non-edible parts of some fruits, can concentrate high levels of valuable bioactive compounds, particularly natural antioxidants (Vinha et al., 2013).

2. Materials and Methods

2.1 Sample Collection and Preparation

All the avocado fruits, variety 'Hass' used in the present study came from an orchard located in the Faro area (Latitude: 37.019°, Longitude: -7.926°). The fruits, a total of 100 at the onset of ripening, were randomly collected and selected by their firmness, absence of mechanical damage and visible decay. Immediately after harvest the fruits were cleaned and prepared according to the requirements of the intended analysis. They were cut open to obtain their edible and non-edible portions (pulp, peel, and seeds, respectively) and stored at 4ºC. Six replicates of each sample were selected and analyzed. All analyzes were carried out over a period of time not exceeding two weeks after harvest.

2.2 Standards and Reagents

2,6-dichlorophenol-indophenol (Tillmans reagent), glacial acetic acid, meta-phosphoric acid, DL-α-tocopherol acetate sodium carbonate, β-carotene, petroleum ether, ascorbic acid, sodium phosphate, aluminium chloride and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol, the Folin-Ciocalteu reagent, sodium hydroxide, sulphuric acid, and gallic acid were purchased from Panreac Química S.L.U. (Barcelona, Spain). All aqueous solutions were prepared with Milli Q filtered water (resistivity >18 MΩ.cm) (Millipore, Bedford, MA).

2.3 Proximate Composition Analysis

Moisture, titratable acidity (TA), total soluble solids (TSS) were evaluated as quality fruit indices. The ash, total protein and total fat contents were also analyzed. A gravimetric assay was performed to evaluate the

physiological weight loss of the avocado fractions (pulp, peel, and seeds). It was calculated by the difference between initial and final weight. A porcelain capsule containing 5 g of each fresh avocado fraction was placed in a stove (WTC binder Klasse 2.0, Tuttlingen, Germany) at 105±1ºC, followed by regular weighing up to a constant weight. Results were expressed in water percentage (%). TA was determined by titrating 5 ml of avocado aqueous extract with 0.1 M NaOH, using phenolphthalein (1%) as indicator. Results were expressed as grams of tartaric acid per 100 g of sample, according to the methodology described by the Association of Official Analytical Chemists (2005). The TSS were quantified using a hand digital refractometer Leica Abbe Mark II (Leica, Buffalo, NY, USA) and expressed as °Brix.

As with all food analysis procedures it is crucial to carefully select a sample whose composition represents that of the food being analyzed and to ensure that its composition does not change significantly prior to analysis. The following methods (AOAC, 2005) were used to determine protein, fat and ash content in stored avocado pulp, peel and seed samples: micro Kjeldahl for protein (N x 5.7) (method 960.52) (Glass Model Pyrex-1); incineration at 550ºC for ash (method 923.03) (PCSIR-Lhr); defatting in a Soxhlet apparatus (J.P.Selecta–Spain) with 2:1 (v/v) chloroform/methanol for lipids (method 920.39C). All experiments were repeated in sextuplicate and the values are presented as mean (±SD).

2.4 Bioactive Compounds Quantification

2.4.1 Extraction and Analysis of Ascorbic Acid

Avocado fruit fractions (5 g) were dissolved in a mixture of 200 ml of water, 5 ml of metaphosphoric acid (30%) and 20 ml glacial acetic acid. The mixture was titrated with Tillmans reagent. Ascorbic acid (expressed as mg/100g (on a FW – fresh weight basis)) was quantified using an analytical validated method published in a previously work (Vinha et al., 2012).

2.4.2 Colorimetric Determination of Tocopheryl Acetate (Vitamin E)

The determination of the vitamin E content in the different constituents of Algarvian avocado fruit followed the procedure described by Amin (2001). From a standard solution of α-tocopherol acetate in 100 ml of methanol, several dilute solutions were prepared by taking 10, 25, 50, 100, 200, 400 µl aliquots of the stock solution and placing them in 25 ml calibrated flasks.

α-tocopheryl acetate was converted into α-tocopherol by transesterification. Standards were prepared by taking

α-tocopheryl acetate was converted into α-tocopherol by transesterification. Standards were prepared by taking 10, 25, 50, 100, 250 and 500 µl portions of stock solution in 25 ml calibrated flasks, adding a drop of sulphuric acid, to catalyze the reaction, and 20 ml of methanol to each, and heating at 90°C in a water-bath for 90 min; within this period, the flask contents were reduced almost to dryness. The end-product of transesterification was dissolved in 15 ml of methanol, and 5.0 ml of NaOH (0.2 M) were added. The absorbance at 526 nm was measured after 10 min of heating in a water-bath at 90±2°C. The experiments were performed in sextuplicate for each avocado fruit fraction (pulp, peel, and seed).

2.4.3 Total Carotenoids Assay

Total carotenoids were extracted according to Akin et al. (2008) with some minor modifications. Briefly, five grams of sample were homogenized using a high-speed homogenizer, at 5000 rpm for 30 minutes (Heidolph, Diax 900, Germany) and then transferred to a separating funnel for extraction with 100 ml of methanol/petroleum ether (1:9, v/v). The petroleum ether layer was then filtrated through sodium sulphate, transferred to a 100 ml volumetric flask and dissolved with petroleum ether. Finally, total carotenoid content was measured spectrophotometrically (Hitachi UV-2800 spectrophotometer) at 450 nm by using an extinction coefficient of 2592. Results were expressed as β-carotene equivalents (milligrams per 100 g of FW).

2.4.4 Total Polyphenolic Content Assay

Total phenolics were determined according to the improved Folin-Ciocalteu method (Zieliski & Kozowska, 2000). Briefly, 5 g of fresh avocado fruit fractions were homogenized by using a homogenizer (model F.60, Falc Instruments, Italy) in water (100 ml) kept at 40°C for one hour and then filtered. The avocado fruit extracts were then resuspended in water and the supernatant (0.5 ml) was mixed with 0.5 ml of Folin-Ciocalteu's solution. The solution was homogenized for 3 minutes and 1 ml of saturated Na_2CO_3 was added. The solution was then incubated for 1 hour in the dark to obtain color development, through the reduction of phosphomolybdic and phosphotungstic acids in alkaline medium. The absorbance readings were measured at 720 nm with an UV-VIS spectrophotometer (Shimadzu UV-2100), using gallic acid (GA) as standard. Total phenol content was expressed as milligrams of GA equivalent (GAE) per 100 grams of fresh fruit weight (mg GAE /100 g^{-1} FW).

2.4.5 Total Flavonoids Content

Flavonoid contents in the aqueous extracts of the pulp, peel, and seeds of avocado fruits were determined using a method described by Soares et al. (2013) with slight modifications. Aliquots of 1 ml of extract solution were mixed with 4 ml of water and 300 µL sodium nitrate 25%. After 5 min incubation at room temperature it was added 300 µl of $AlCl_3$ reagent (10%), and left to react for one minute before adding 2 ml of sodium hydroxide and 2.4 ml of water. The absorbance was recorded at 510 nm in a BioTek Synergy HT microplate reader (GENS5). The flavonoid contents were express in milligrams per 100 grams of FW.

2.5 DPPH• Radical-Scavenging Activity

Pulp, peel and seeds of avocado aqueous extracts (300 µl) were mixed with 2.7 ml of an ethanolic solution containing DPPH• (2,2-diphenyl-1-picrylhydrazyl radical) in a concentration of 6 $x10^{-5}$ M. The mixture was shaken vigorously and left to stand in the dark until stable absorbance readings at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH• discoloration using the equation: % RSA = [(ADPPH• – AS)/ADPPH•] x 100, where AS represents the absorbance of the sample solution extract with DPPH•· and ADPPH• is the absorbance of the DPPH• solution.

2.6 Statistical Analysis

A completely randomized design was used, with six replications. Statistical analysis was performed using SPSS v. 21 (IBM Corp., Armonk, NY, USA). Data of all chemical analysis were expressed as mean ± standard deviation. The independent samples T-test or Analysis of Variance (ANOVA) were used to assess the statistical differences among means followed, in the case of ANOVA, by Tukey's HSD post-hoc test for multiple comparisons. Pearson correlation tests were used to ascertain the existence of linear relationships between the contents of bioactive compounds and antioxidant activity. The level of significance for all hypothesis tests (p) was 0.05.

3. Results and Discussion

As previously referred, the objectives of this study were to characterize the Algarvian avocado in terms of food and potential source of bioactive compounds for the food and cosmetics industries. The results obtained for the fruit physicochemical parameters are presented in Table 1.

Table 1. Physicochemical parameters of the different fractions of the Algarvian avocado variety 'Hass'. Moisture, proteins, ash and fat are expressed in percentage. The Total Soluble Solids in °Brix and the acidity in mg of tartaric acid equivalents /100g FW

	Fraction of the Algarvian avocado var. 'Hass'		
Parameter**	Pulp*	Skin*	Seeds*
Moisture (%)	70.83±3.53^a	69.13±2.58^b	54.45±2.33^c
Ash (%)	1.77±0.16^a	1.50±0.08^b	1.29±0.03^c
Proteins (%)	1.82±0.07^a	1.91±0.08^a	2.19±0.16^b
Fat (%)	43.5±4.62^a	2.20±1.65^b	14.7±0.32^c
Total Soluble Solids (°Brix)	6.68±1.02^a	3.01±2.03^b	3.54±1.97^b
Acidity	1.07±0.02^a	2.05±0.24^b	2.67±0.17^c

As shown in Table 1, *Values represented as mean±standard deviation obtained from six measurements; **A letter is used to express the result of the comparison between the different fractions. Different letters indicate significant statistical differences (95% significance).

The moisture content is one of the most important indices evaluated in foods, especially fruits. It is a good indicator of their economic value because it reflects solid contents and serves to assess its perishability. The results indicate that the Algarvian avocado pulp has a higher water content (70.83%) , followed the skin

The moisture content is one of the most important indices evaluated in foods, especially fruits. It is a good indicator of their economic value because it reflects solid contents and serves to assess its perishability. The results indicate that the Algarvian avocado pulp has a higher water content (70.83%) , followed the skin (69.13%) and seed (54.45%). The fat and ash quantified in pulp were significantly superior to those found in the skin. The seed was the part of the fruit that had higher amounts of total protein (2.19%) and lowest ash content (1.29%), nevertheless, relative to its fat content, showed higher percentages compared to those found on the exocarp. According to Hernández-Muñoz et al. (2006) the total acidity is a measure of the organic acid content. The predominant acid found in avocados is tartaric acid although, theoreticaly, every species capable of donating a proton, including fatty acids, also contribute to the total acidity of the fruit (Omar et al., 2012). Acidity and soluble solids content are the common quality attributes that are associated with the maturity index of

agricultural products, especially fruits. The total acidity tends to decrease during the ripening period as a result of the breathing process or conversion into sugars. In the period of maturation of the fruit there is an increase in metabolic activity and organic acids are, par excellence, a source of energy reserve of the fruit through the Krebs cycle. In the case of the mature Algarvian avocado, the seed has higher acidity than the skin or pulp. The acidity of the pulp was found to be superior to that exhibited by 'Hass' avocados of American origin (0.04±0.01% citric acid) (Arias et al., 2012). In any case Algarvian 'Hass' avocados may be considered a non acidic fruit.

Among the various components of fruit, the total soluble solids (i.e., the percentage of solids that are dissolved in the matrix of the food) in °Brix, have a primary role in their quality due to the influence on thermophysical, chemical and biological properties. It is also a parameter which tends to increase with the progress of ripening due to the biosynthesis of the plant and degradation of polysaccharides. As expected, given that this physico-chemical parameter represents one of the best ways to evaluate the degree of sweetness of the fruit, and the fruit pulp is the only edible part of the avocado, the total soluble solids are higher in the pulp. This parameter follows a trend that is opposite to acidity. Nevertheless the content of soluble solids, although superior to those reported for 'Hass' avocados of American origin (5.1±0.1°Brix) (Arias et al., 2012), can be considered low, favoring the consumption of the Algarvian avocado *in natura*. Superior values of TSS have been reported for 'Hass' avocados from New Zeland (~9° Brix) (Burdon et al., 2007).

The characteristics of a fruit depend on the cultivar, the edaphoclimatic conditions of the region of provenance, ripeness and storage conditions (Ahmed et al., 2010). Tango et al. (2004) studied 24 varieties of avocado, and found levels for moisture and fat in the pulp of 'Hass' variety fruits of 57.3% and 31.1%, respectively. These values are significantly lower than those found in the Algarvian avocado studied here. Regarding the avocado seeds, Olaeta et al. (2007) observed higher protein concentrations and ash, compared with those recorded in this study (3.18% and 1.51%, respectively). Lu et al. (2009) on the other hand, reported a value of 25% fat for the pulp of 'Hass' avocados cultivated in California. The Algarvian avocado develops mainly during the winter because during the rest of the year the orchards in the Algarve are subjected to water stress. This is an important factor to justify the results presented in Table 1.

There is already evidence that the ingestion of fruits confers protection against human chronic diseases, neurological disorders and some types of cancer (Middleton et al., 2000; Pandey & Rizvi, 2009; Hamid et al., 2010). These properties are assigned to the presence of significant levels of bioactive antioxidant compounds in fruits. For this reason, those molecules are attracting a growing interest from the scientific community. During the last decades, ample evidence of the benefits of avocado on health has been gathered (Yasir et al., 2010; Al-Dosari, 2011). This promoted their consumption, stimulating also the research about their pharmacological potential. The maturation of any fruit promotes an increase of bioactive compounds (Arancibia-Avila et al., 2008). Among the different secondary metabolites with antioxidant properties, phenolics, flavonoids and carotenoids are the most cited. The levels of these compounds, as well as those of the vitamins C and E, found in the Algarvian avocado are presented in Table 2.

Table 2. Concentration of bioactive compounds present in different Algarvian avocado 'Hass' var. fruit fractions

	Avocado fraction var. 'Hass'		
Bioactive compound **	Pulp*	Skin*	Seed*
Total Phenolics	410.2±69.0^{b}	679.0±117.0^{a}	704.0±130.0^{a}
Flavonoids	21.9±1.0^{b}	44.3±3.1^{a}	47.9±2.7^{a}
Carotenoids	0.815±0.201^{b}	2.585±0.117^{a}	0.966±0.164^{b}
Vitamin C	1.2±0.7^{c}	4.1±2.7^{a}	2.6±1.1^{a,c}
Vitamin E	5.36±1.77^{a}	2.13±1.03^{b}	4.82±1.42^{a}

As shown in Table 2, *Values represented as mean±standard deviation mg/100g FW obtained from six measurements; **A letter is used to express the result of the comparison between the different fractions. Different letters indicate significant statistical differences (95% significance).

The results reveal that is in the avocado seed that the highest levels of total phenolics and flavonoids are found. This agrees with the results reported for avocados cultivated in Mexico (Wang et al., 2010). The skin of the fruit had the highest carotenoid content, as expected, since this tissue is usually the fraction were these phytochemicals

are concentrated. Recently a study proved that the composition of carotenoids and vitamin E in fruits is affected by several factors, including the degree of maturation and edaphoclimatic (Arancibia-Avila et al., 2008). Significant differences were found in the levels of carotenoids and vitamin E in 'Hass' avocados cultivated in four different Californian counties. It was concluded in the same study that the levels of carotenoids in the fruit pulp increased with the fat present in it and that the xanthophylls, in particular lutein and cryptoxanthin, were the predominant phytochemicals of this group, contributing approximately to 90% of the total carotenoids present in the 'Hass' avocado (Lu et al., 2005). When one compares the contents of bioactive compounds of the Algarvian fruit with those of other fruits produced in different parts of the globe, it may be noted that it has levels of phenolics in the pulp comparable to those found in Mexican 'Hass' avocados (4.9±0.7 mg GAE/g FW), inferior levels in the skin (12.6±0.3 mg GAE/g FW) and seeds (51.6±1.6 mg GAE/g FW) (Wang et al., 2010) while possessing comparable levels of flavonoids (26.36 QE/100 g FW) (Rodríguez-Carpena et al., 2011). The phenolic levels are also superior to those reported for the same fruit of Turkish provenance (1.20±0.02 g/kg FW) (Golukcu & Ozdemir, 2010). The content of carotenoids is inferior to that found in Californian avocados (42.2 μg/g) (Lu et al., 2009) and higher than that found in the corresponding fractions of Mexican 'Hass' avocados ((7.1±0.6 μg/g (pulp), 15.2±2.7 μg/g (skin), 6.3±0.9 μg/g (seed)) (Wang et al., 2010). Furthermore the Algarvian avocado has superior levels of carotenoids in the pulp than the 'Hass' avocados cultivated in New Zeland (~5.2 μg/g), but inferior levels in the skin (~50 μg/g) (Ashton et al., 2006). The mesocarp of the Algarvian avocado presented higher levels of vitamin E, with a value that is statistically similar to that found in the seeds and above that found in the skin. The amount of this vitamin found in the pulp is comparable to that found in avocados grown in Brazil (6.4 mg/ 100g) (Salgado et al., 2008) but superior to that of avocados from california (27 μg/g) (Lu et al., 2009). The concentration of ascorbic acid is inferior to that reported for Californian avocados (17.3 mg/100g) (USDA, 2011).

Overall these results also demonstrate the potential of the non-edible parts of the avocado as a source of bioactive compounds. The skin of the Algarvian 'Hass' avocado contains 59% of the carotenoids and the seeds 39% of total phenolic compounds and 42% of the flavonoids present in the fruit. Instead of being wasted as trash, fruit skin could constitute an inexpensive source of carotenoids in the dermocosmetic and food industries. Indeed the avocado is the fruit with the highest content of carotenoids in the exocarp. The carotenoid compounds are known to exert a protective action against cell damage caused by UV rays and pollution, which make them an essential ingredient of several dermatological formulations. Additionally the carotenoids, phenolics and flavonoids are known to prevent the risk of developing certain diseases related to age, such as premature aging, cancer and heart disease (Hidalgo et al., 2010). Both the skin and the seeds can also be harnessed as a source of these compounds to use as food additives (Ayala-Zavala et al., 2011). Remarkably the skin and seeds of avocado have higher levels of these compounds than those that exist in many other fruits and vegetables such as apple (*Malus domestica*), banana (*Musa cavendish*), tomatoes (*Lycopersicum esculentum*) or red cabbage (*Brassica oleracae var. botrytis*) just to name a few (Marinova et al., 2005; Lin & Tang, 2007; Sulaiman et al., 2011; Vinha et al., 2013).

Consistent with the fact that they contain higher levels of bioactive antioxidant compounds, it was found that the avocado seeds also exhibit higher, and statistically different, values of *in-vitro* antioxidant activity (measured in this work through the ability to scavenge the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•)), Figure 1.

These results differ slightly from those reported in the literature since both Wang et al. (2010) and Rodríguez-Carpena et al. (2011) showed that the skin had superior antioxidant activity. In fact it turns out that both the skin and the seeds of avocado fruit are very rich in antioxidant compounds however the seed has greater content of flavonoids and phenolic compounds while the skin is richer in carotenoids. In general, the contribution of vitamin C to the total antioxidant capacity of extracts varies with the type of fruit. In fact, vitamin C due to its hydrophilic character is unique among the vitamins present in the avocado matrix, the majority of which, namely vitamins A, D and E, are all liposoluble. It is well known fact that the bioactive compounds do not all have the same antioxidant activity, thus, an increase in the level of a compound does not mean a proportional increase of antioxidant activity of the matrix (Sanjust et al., 2008). Furthermore for a complex extract, as the one in question, it is also necessary to take into account the synergistic or antagonistic effects among the various compounds present, which makes not only the antioxidant activity dependent of the concentration of each compound but also of the interaction between different compounds, antioxidants or not. Perhaps this is why when the concentration of the extracts doubles, the antioxidant activity exhibited by the pulp increases but remains unaltered in case to the skin and seeds.

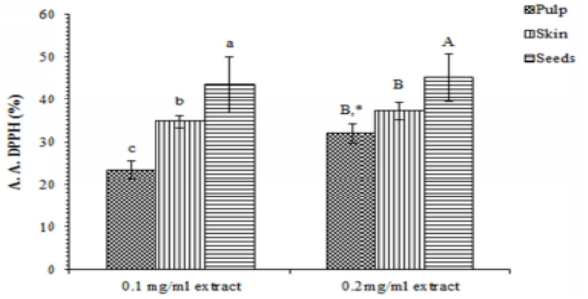

Figure 1. Antioxidant activity (A.A.) of aqueous extracts obtained from the various avocado fractions on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•). The symbol "*" indicates the existence of significant statistical

differences ($p<0.05$) among the antioxidant activity exhibited by the two aqueous extracts of the same fraction. Identical letters signalize extracts that exhibit the same antioxidant activity

Most studies have demonstrated a linear correlation between total phenolic content and the antioxidant activity evaluated by different methodologies in fruits and vegetables (Mahattanatawee et al., 2006; Corral-Aguayo et al., 2008). Regression analyses were performed to correlate the antioxidant activity of avocado samples with the antioxidants quantified in the avocado tissues (Table 3).

Table 3. Correlation among the contents of bioactive compounds and DPPH• antioxidant activity

Extract matrix	Flavonoids x DPPH•	Phenolics x DPPH•	Carotenoids x DPPH•	Vitamin C x DPPH•	Vitamin E x DPPH•
Pulp	-0.436	-0.094	-0.314	0.238	0.123
Skin	0.678	-0.430	-0.132	0.220	-0.880
Seeds	-0.506	0.715	0.703	0.011	0.641

Considering all the different antioxidant compounds, a good correlation was found between total phenolic content determined by the Folin-Ciocalteu and flavonoids contents and DPPH radical scavenging capacity ($r = 0.783$) and ($r = 0.820$), respectively. However, analyzing the fruit fractions separately, good positive correlations were only found for the contents of carotenoids, total phenolics and vitamin E and antioxidant activity exhibited by the seeds extracts and the contents of flavonoids in the case of the skin extracts.

4. Conclusions

Despite not being native of the region, the Algarvian avocado variety 'Hass' is a fruit with excellent physical and chemical characteristics, with moisture, protein, fat and ash comparable or superior to 'Hass' avocados from Mexico and California. Its levels of bioactive compounds are also comparable in the different constituent fractions of the fruit. Thus, for every 100 g of Algarvian avocado var. 'Hass', its edible portion (pulp) has, on average, 410.2±69.0 mg of total phenolics, 21.9±1.0 mg of flavonoids, 0.815±0.201 mg of carotenoids, 1.2±0.7 mg de vitamin C and 5.36±1.77 mg of vitamin E. The non-edible parts, i. e., the skin and seeds were found to have an average of 679.0±117.0 mg total phenolics, 44.3±3.1 mg of flavonoids, 2.585±0.117 mg of carotenoids, 4.1±2.7 mg of vitamin C and 2.13±1.03 mg of vitamin E (skin) and 704.0±130.0 mg of total phenolics, 47.97±2.69 mg of flavonoids, 0.966±0.164 mg of carotenoids, 2.6±1.1 mg of vitamin C and 4.82±1.42 mg of vitamin E (seeds). In accordance with the higher levels of bioactive compounds the extracts obtained from the seeds and skin of avocado

presented higher antioxidant activity against the DPPH• (43% and 35%, respectively) compared to that exhibited by the pulp (only 23%). The fact that the non-edible parts of the fruit (skin and seeds) contains such high levels of carotenoids, flavonoids and phenolics makes the idea of their exploitation, as a cheap source of these compounds in the food industry and dermo-cosmetics, very appealing. The mass of byproducts obtained as a result of processing tropical exotic crops, such as that of avocado, may approach or even exceed that of the corresponding edible part affecting the economics of growing of these crops.

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Effects of Bioactive Component of Kiwi Fruit and Avocado (Fruit and Seed) on Hypercholesterolemic Rats

[1]Manal M.S.M. Shehata and [2]Sahar S.A. Soltan

[1]Department of Food Science, Faculty of Agriculture, Zagazig University, Zagazig, Egypt
[2]Department of Home Economics (Nutrition and Food Science),
Faculty of Specific Education, Fayoum University, Egypt

Abstract: This study evaluated the effect of kiwi fruit and avocado (fruit and seed) on diet-induced hypercholesterolemia in rats. The results showed that avocado seed content was the highest in phenolic compounds (285.43 mg/100g GAE); flavonoids (3.21mg/100g CE) and soluble dietary fiber (38%) followed by avocado and kiwi fruits. Kiwi fruit is rich in ascorbic acid (15.52 mg/100g). Hypercholesterolemia was induced using a diet containing 1% cholesterol and 16%fat. Sixty six male of Albino rats weighing 115-120g were randomly distributed into eleven groups of six rats each-Group 1: control rats fed on the standard diet, group 2 rats fed on hypercholesterolemic diet, while the other groups of rats fed hypercholesterolemic diet supplemented with kiwi fruit and avocado (fruit and seed) (10%, 20% and 30%).The present study showed that 1% cholesterol and 16% fat administration for 6 weeks caused a significant increase $P<0.05$ in total cholesterol and triglyceride in both serum and liver. In serum, the levels of total cholesterol, triglyceride and LDL-C significantly decreased for the groups fed kiwi fruit and avocado (fruit and seed) in comparison with the hypercholesterolemic group (HC group). The activities of AST and ALT enzymes decreased significantly for the groups fed the kiwi fruit and avocado (fruit, seed) in comparison with the HC group. Atherogenic index (AI) increased significantly compared to control group. Regarding liver tissue, the levels of total cholesterol and triglyceride decreased significantly for the kiwi fruit and avocado (fruit, seed) fed rats compared to the HC group. The liver content of reduced glutathione increased significantly in comparison with the hypercholesterolemic group. In this study, the lowering effect of avocado seed on lipid profiles in serum and liver was more observable than that of avocado fruit or kiwi fruit. The results suggest that consumption of kiwi fruit and avocado (fruit and seed) might have some cardiovascular protective properties and beneficial effects on atherosclerosis, CVD risks in hypercholesterolemic rats. So, we recommended consuming avocado seed because the avocado seed have strong antioxidant activity and lower effect of lipid profile.

Key words: Kiwifruit · Avocado fruit · Avocado seed · Lipid profiles · Diet-hypercholesterolemia · Risk factors · Cardiovascular disease

INTRODUCTION

Hypercholesterolemia is a lipoprotein metabolic disorder characterized by high serum low density lipoprotein and blood cholesterol. It is major risk factors in the development and progression of atherosclerosis that leads to cardiovascular disease [1]. Hypercholesterolemia is a major problem to many societies especially the health professionals because of the close correlation between cardiovascular diseases and lipid abnormalities [2, 3]. Dietary factors such as continuous ingestion of high amounts of saturated fats and cholesterol are believed to be directly related to hypercholesterolemia and susceptibility to atherosclerosis [4]. Clinical trials have demonstrated that intensive reduction of plasma low density lipoprotein (LDL-C) levels could reverse atherosclerosis and decrease the incidence of cardiovascular diseases [5].

Corresponding Author: Sahar S.A. Soltan, Department of Home Economics (Nutrition and Food Science), Faculty of Specific Education, Fayoum University, Egypt.

Plants in general and fruits particularly have several compounds with antioxidant properties, which include ascorbic acid, carotenoids and polyphenols. Increased consumption of fruits protects cardiovascular diseases [6, 7]. Kiwi fruit (*Actinidia deliciosa planch*) is one of the most popular fruits worldwide and is cultivated in many countries, such as New Zealand, Italy, Japan, Greece and France [8]. There are many Kiwi fruit cultivars and the most known is "Hayward". "Biden" is less spread than "Hayward" and both cultivars belong to the Actinidia deliciosa species and are known for good taste [9-11]. Kiwi fruit is a highly nutritional fruit due to its high level of vitamin C and its strong antioxidant including carotenoids, lutein, phenolics, flavonoids and chlorophyll [12]. Kiwi fruit is a rich source of vitamins E, fructose, galactose and minerals, its contains isoflavones and flavonoids which are important phytochemical in kiwi extract and represent the major class of phytosterogen, which has an important function as anti-carcinogenic, neuroprotective and cardio protective activity [13, 14]. Recent studies have shown that kiwi fruit have antioxidant [9], cardiovascular protective [15]. Extracts of kiwi fruit inhibit cancer cell growth and exhibit cell

Chemicals: Folin-Ciocalteu reagent, methanol, 2, 2 diphenyle-1-picryldrozyl (DPPH), Gallic acid, NaCo3, AlCl3,Naok tartarate and purified cholesterol were purchased from sigma- Chemical Company, USA. Kits for blood analysis were purchased from Biodiagnostic Co. Dokki, Giza, Egypt. All other reagents used were analytical grade. All solvent used (isopropanol and methanol) were obtained from El-Goumhouria Co. Cairo, Egypt.

Methods:

Preparation of Fruit and Seed Extract: The kiwi fruit, avocado (fruit and seed) were extracted according the method of Biglari *et al.* [30], this method modified to obtained the maximum yield as follows: 500 gram fruits and seed was pitted, cut to small pieces and dry-blended for 3min. The blended fruits were extracted with 1 liter methanol: water (50:50 v/v), at room temperature 25°C for 10 hours using an orbital shaker. The extracts were then filtered and centrifuged and the supernatant was concentrated under reduced pressure at 40°C using rotary evaporator to obtain the methanol crude extracts of the fruit and seed. The extracts were kept in dark glass bottles and stored at-18°C until used.

Avocado (*Persea americana* Mill) is an important commercial tropical fruit. Avocado fruit content high levels of bioactive compounds and including vitamin E, Ascorbic acid, carotenoids and soluble phenolics [18]. It is contains one to two times more protein than any other fruit, is high in manganese, phosphorous, iron and potassium, but low in sodium and also contains vitamin C, â-carotene, thiamin, riboflavin, nicotinic acid and folate [19]. Avocado is a good source of the essential linoleic acid. The amount of simple sugars in the avocado fruit is low, but in contrast, it contains appreciable levels of dietary fiber and is the highest in fiber among fruits [20]. In the folk medicine of Latin America and Africa, it has been used as a remedy for hypertension [21], renal diseases [22] and diabetes [23, 24] and for antipyretic and analgesic purposes [25]. Some studies in rats have demonstrated the hypotensive [26, 27], antioxidant [28] and hypocholesterolemic properties [29] of the extract of *Persea americana* leaves, partially confirming the popular belief. The present study was designed to determination bioactive component and hypocholesterolemic effect of kiwifruit and avocado (fruit and seed) in rats.

MATERIALS AND METHODS

Materials: The mature fresh avocado (*Persea Americana* Mill.) and kiwi fruit (*Actinidia deliciosa*) Hayward strain were purchased from local markets in Egypt.

Determination of Crude Fibers, Dietary Fiber (Insoluble and Soluble) and Ascorbic Acid: Crude fibers, dietary fiber (insoluble and soluble) and Ascorbic acid in kiwi fruit, avocado fruit and seed were analyzed by the method of AOAC [31].

Determination of Total Phenolic Compounds: Total phenolics compounds of kiwi fruit and avocado (fruit and seed) were determined spectrophotometer using Folin-Ciocalteu colorimetric method [32]. Briefly 5ml of distilled water, 0.5-1.0 ml of each sample of extract, 1.0 ml of Folin-Ciocalteu reagent was added to a 25ml volumetric flask. The contents were mixed and allowed to stand at room temperature for 5-8 min. Then 10 ml of 7% $NaCO_3$ solution was added, followed by the addition of distilled water filled to volume. Solutions were mixed and allowed to stand at room temperature for 2hours. Sample aliquots were filtered through a Whatman 0.54 in polytetrafluoro ethylene filter prior to the determination of total phenols concentration using a spectronic 2000 spectrophotometer monitoring 750nm. Total phenoilc compounds was standardized against Gallic acid and expressed as milligram per liter of Gallic acid equivalents (GAE).

Determination of Total Flavonoids: Total flavonoids content of kiwi fruit and avocado (fruit and seed) extracts were determined by using spectrophotometer according to the method described by Khatiwara *et al.* [33]. Aliquots

extract solution 1 ml, were taken and made up the volume 3ml with methanol, then 0.01 ml AlCl3 (10%), 0.1 ml Na k tartarate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at 415 nm was recorded after 30 min of incubation. A standard calibration was generated at 415 nm using the concentration of flavonoid in the test sample were calculated from calibration plot and expressed as mg quercetin equivalent/g sample.

Identification of Individual Phenolic Compounds by High Performance Liquid Chromatography (HPLC): Phenolic compounds in kiwi fruit and avocado (fruit and seed)were determined by HPLC according to the method by Goupy *et al.* [34] as follows: 5g of samples were mixed with methanol and centrifugated at 1000 rpm for 10 min and the supernatant was filtered through 0.2 Mm Milliporemembrane filter than 1-3 ml was collected in avail for injection into HPLC Hewllet Puckered (Series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet UV detector set at 280 nm and quaternary HP pump (series 1100) packed column Hypesil BDs-C18 4.0 X 250 nm was used to separation phenolic compound. The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. Phenolic acid standard from Sigma Company were dissolved in mobile phase and injected into HPLC. Retention time and peal area were used to calculation of phenolic compounds concentration by data analysis of Hewllet software, Germany.

Identification of Individual Flavonoids by HPLC: Flavonoids compounds were determined by HPLC according to the method of Mattila *et al.* [35] as follow: 5g of sample were mixed with methanol and centrifuged at 1000 rpm for 10 min and the supernatant was filtered through a 0.2 µm Millipore membrane filter than 1-3 ml was collected in a vial for injected into Hewllet Puckered (Series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet UV detector set at 330 nm and quarter HP pump (series 1050).The column was used to separate flavonoid was Zorba X ODS 5 µm (4.6 x 250 nm). The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. Flavonoid standard from Sigma Company were dissolved

Determination of Antioxidant Activity Using 2,2-diphenyle-1-picrylhydrazyl (DPPH) Radical Scavenging Method: Antioxidant activity of fruits and seed was determined using the stable radical (DPPH) according to Brand-Williams *et al*. [36]. Aliquot of 0.1 ml methanol solution containing different concentration from kiwi fruit and avocado (fruit and seed) was added to 3.9 ml of 6 $x10^{-5}$ methanolic solution of freshly prepared DPPH. After 30 min incubation at room temperature, the absorbance was read at 515 nm by Perkin Elr spectrophotometer for all tested samples. DPPH solution without fruit and seed extracts were used as control. Percentage inhibition % of DPPH free radical was calculated according to the following equation:

$$\% \text{ Initiation} = \frac{\text{Absorbance control-Absorbance sample}}{\text{Absorbance control}} \times 100$$

$$\text{Antiradical efficiencies (AE)} = \frac{1}{EC_{50}}$$

EC_{50} extraction concentration providing 50% inhibition of the DPPH.

Animals: Sixty-six male Albino rats weighing 115-120g were purchased from animal house of El-Salam Farm, Giza, Egypt. The animals were placed in individual cages at 25°C under a 12 h light/dark cycle. Water was provided *ad libitum*. After one week of acclimatization, the rats were divided randomly into eleven groups of six rats in each (n = 6) and treated as follows:

Group 1(G1): Rats fed basal diet assigned as normal control group. Basal diet was preparing according to Reeves *et al.* [37].

Group 2 (G2): Rats fed basal diet contained 1% cholesterol + 16% fat and 0.2% cholic acid and assigned as a hypercholesterolemic (HC) rats according to Harnafi *et al.* [38].

Groups 3, 4 and 5: Rats fed HC diet supplemented with10%, 20% and 30% kiwi fruit.

Groups 6, 7 and 8: Rats fed HC diet supplemented with 10%, 20% and 30% avocado fruit.

Flavonoid standard from Sigma Company were dissolved in mobile phase and injected into HPLC. Retention time and peal area were used to calculation of flavonoid compounds concentration by data of Hewllet Packared software. Germany.

Groups 9, 10 and 11: Rats fed HC diet supplemented with 10%, 20% and 30% avocado seed. Feed intake was measured daily for each rat. Body weight was measured at baseline and at 6 weeks and feed efficiency ratio

(FER) was calculated. At the end of experimental period (6 weeks) animals were sacrificed after overnight fasting. The serum was separated by allowing blood samples left for 15 minutes at temperature of 25°C then centrifuged at 300 rpm for 20 minutes, then kept in plastic vials at -20°C until analysis. Main of organs was carefully separated and washing with cold saline and dry on filter paper and the weight was recorded.

Biochemical Analysis: Serum was analyzed for the following biochemical parameter: total cholesterol by the method of Richmond [39], HDL-cholesterol by Burstein *et al.* [40], triglyceride by Jacobs and Vander mark [41] and AST and ALT by Reitman and Frankel [42]. Calculation of LDL-Cholesterol fraction and atherogenic index (AI) and HTR ratio involves equations developed by Friedewald *et al.* [43]:

$$\text{Atherogenic index (AI)} = \frac{\text{(Serum total cholesterol- HDL-c)}}{\text{HDL-c}}$$

$$\text{HTR ratio} = \frac{\text{HDL-c}}{\text{TC}} \times 100$$

Extraction and Determination of TG and TC and GSH in Liver Tissue: At the end of the experimental, Liver was removed, rinsed in ice chilled normal saline and blotted on filter paper and then tissues were cut into small portion and stored at - 20°C before use. Extraction of liver analysis of total cholesterol and triglycerides was carried out according to the method by Hostmark [44]: 1g of liver portion from each rat was homogenized in 10 ml isopropanol. The liver homogenate was allowed to stand for 48 h at 4°C. The mixture was centrifugated 15 min at 2500 rpm and the supernatant was used for lipid analysis. Total cholesterol and triglyceride were quantified using enzymatic as described above. Glutathione reduced activity (GSH) of liver was determined according to the method by Beulter *et al.* [45].

Statistical Analysis: The results obtained were analyzed using SPSS program (version 17.0) and expressed as mean and standard deviations (SD). Statistical significance ($p<0.05$) among the groups were determined by one-way ANOVA followed by Duncan's multiple range test according to the method by Bailey [46].

RESULTS AND DISCUSSION

Bioactive component in kiwi fruit and avocado (fruit and seed) was illustrated in Table 1. The data in Table 1 revealed that avocado fruit was the highest in crude fiber (12.84%) followed by kiwi fruit (11.22%) and avocado seed (9.42%). Also avocado fruit and seed have higher dietary fiber (6.56 and 7.61mg/100g) and insoluble dietary fiber (64 and 62%) and soluble dietary fiber (36 and 38%) compared to kiwi fruit (3.7 mg/100g, 56.2% and 24.1%) respectively. Also data in Table 1 showed the avocado fruit and seed have higher content flavonoids and lower level of ascorbic acid compared to kiwi fruit. These results agreement with Reyes-Caudello [47] reported avocado seed have higher total dietary fiber (39.9 and 36.9%). Park *et al.* [48] reported the amount ascorbic acid in kiwi fruit ranged from 6.56 to 152 mg/100g.

The yield, total phenolic compounds, efficient concentration and antiradical efficiencies of kiwi fruit and avocado (fruit and seed) are shown in Table 2. The maximum yield percentage was obtained for kiwi fruit (18.1%) followed by avocado fruit (14.6%) and avocado seed (12.5%). The results showed the highest concentration of phenolic compound was obtained of avocado seed extract (285.43 mg/100g GAE) followed by avocado fruit (259.15 mg/100g GAE) and kiwi fruit (258.55 mg/100g GAE). These results indicated that the kiwi fruit and avocado (fruit and seed) has higher levels of phenolic compounds. These results are accordance with those obtained by Gorinstein *et al.* [49], who reported that phenolic compound of kiwi fruit extract was higher. Wang *et al.* [50] indicated that avocado seed content the highest total phenolic content and antioxidant capacities whereas the pulp had the lowest. Phenolic content of avocado seed was 88.2 mg/g compared to flesh 1.3 GAE [51]. Among the sample investigated, avocado seed extract showed the highest antiradical efficiency (0.122) and the lowest amount of extract required to scavenge 50% DPPH radical (8.21 µg sample/µg DPPH) (Table 2). The amount of kiwi fruit, avocado fruit and avocado seed extracts required to scavenging 50% of DPPH radical was 30.12, 24.73 and 8.21 µg sample/µg DPPH respectively. Soong and Barlow [51] reported antioxidant activity of avocado seeds was much higher than edible portion.

Data in Table 3 showed that remaining DPPH % of extracts of kiwi fruit and avocado (fruit and seed). The results showed concentration 5µg sample/µg DPPH, the remaining DPPH% was 91.6%, 89.9% and 69.5 of kiwi fruit, avocado fruit and avocado seed extracts. When the concentration increased to 15µg sample/µg DPPH the remaining percent of DPPH decreased to 75.5%, 70.2% and 10%, respectively for kiwi fruit, avocado fruit and avocado seed. Antioxidant activity of avocado seeds was much higher than fruit and kiwi fruit. The effects of antioxidant of avocado seed on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity [52]. Strong activity of

Table 1: Crude fiber, flavonoids and ascorbic acid in kiwi fruit and avocado (fruit and seed).

Bioactive component	Kiwifruit	Avocado fruit	Avocado seed
Crude fiber %	11.22	12.84	9.42
Dietary fiber mg/100g	3.7	6.56	7.6
Insoluble dietary fiber %	56.2	64	62
Soluble dietary fiber %	24.1	36	38

avocado seed may be due to higher content phenolic compounds and flavonoids compounds. Other researchers have found that strong correlation between antioxidant activity as assessed by DPPH and total phenolics [53].

Data in Table 4 showed identification of phenolic

Table 1: Crude fiber, flavonoids and ascorbic acid in kiwi fruit and avocado (fruit and seed).

Bioactive component	Kiwifruit	Avocado fruit	Avocado seed
Crude fiber %	11.22	12.84	9.42
Dietary fiber mg/100g	3.7	6.56	7.6
Insoluble dietary fiber %	56.2	64	62
Soluble dietary fiber %	24.1	36	38
Total flavonoid mg/100g	1.68	2.96	3.21
Ascorbic acid mg/100g	15.52	9.37	5.24

Table 2: Yield, total phenolic compounds, efficient concentration and antiradical efficiencies of kiwi fruit and avocado (fruit and seed).

Extracts	Yield g/100g	Total phenolic mg/100g	Efficient concentration EC_{50}	Antiradical efficient AE
Kiwi fruit	18.1	258.55	30.12	0.033
Avocado fruit	14.6	259.15	24.73	0.040
Avocado seed	12.5	285.43	8.21	0.122

Table 3: Scavenging activity of methanol extract of Kiwi fruit and avocado (fruit and seed) determined by the scavenging of DPPH radial.

Concentration of methanol extract µg sample/µg DPPH	% Scavenging activity of kiwi fruit	% Scavenging activity of avocado fruit	% Scavenging activity of avocado seed
5	91.6	89.9	69.5
10	80.1	83.6	40
12	79.6	75.2	25
15	75.5	70.2	10

Table 4: Phenolic compounds of kiwi fruit and avocado (fruit and seed) (ppm).

Phenolic compounds	Kiwi fruit	Avocado fruit	Avocadoseed
Pyrogallol	224.51	217.57	226.93
Syringic	..	..	768.59
Protocatechuic	13.85	23.34	54.32
Vanillic	4.32	3.88	50.89
Chlorogenic	57	..	154.71
Catechol	5.33	21.36	28.94
Caffeine	2.28	..	5.58
Catechin	48.62	47.78	196.20
Ferulic	..	2.06	14.60
Cinnamic	3.41	..	0.35
Coumarin	3.86	0.99	..
Ellagic	..	73.41	..

Table 5: Falvonids compounds of kiwi fruit and avocado (fruit and seed)(µg/100g).

Flavonid compounds	Kiwi fruit	Avocado fruit	Avocado seed
Rutin	..	82.03	53.21
Rosmarinic	901.80	60.71	114.19
Quercitrinic	..	87.40	112.79
Quercitin	..	222.24	61.98
Narengnin	66.63	-	..
Hesperetin	..	176.52	54.62
Apignen	..	..	84.36
Kampferol	..	60.77	..
Hespererctin	826.20	..	387.52

avocado seed may be due to higher content phenolic compounds and flavonoids compounds. Other researchers have found that strong correlation between antioxidant activity as assessed by DPPH and total phenolics [53].

Data in Table 4 showed identification of phenolic compound of kiwi fruit and avocado (fruit and seed) by HPLC. Phenoilc acid (ppm) was the following: pyrogallol (224.51, 217.57 and 226.93ppm); protocatechuic (13.85, 23.34 and 54.32 ppm); vanillic acid (4.32, 3.88 and 50.89 ppm); catechol (5.33, 21.36 and 28.94 ppm); catechin (48.62, 47.78 and 196.20 ppm). Syringic was only detected in avocado seed. Ellagic acid only detected in avocado fruit. The major concentration of phenolic acid was present in avocado seed. These results are in line with those obtained by Park *et al.* [48], who reported that the protocatechuic of two type kiwi was (23.4 and 25.7 mg), vanillic (6.18 and 4.56) and caffeic (45.3 and 17.1 ppm). Rodriquez-Carpena *et al.* [54] reported that phenolic substances are widely distributed in flesh and seed avocado (caffeic acid, P-Hydroxy benzoic, chrouogenic, ferulic and epicatichin).

Identification of flavonoid compounds of kiwi fruit and avocado (fruit and seed) are summarized in Table 5. Rosmarinic and hespererdrin was the major flavonoid in kiwi fruit (901.80 and 826.20 µg/100g) and avocado seed (114.19 and 387.52 µg/100g). Rutin, quercitrnic and quercitin was detectable in avocado fruit and seed and not detectable in kiwi fruit. Apignen was only detectable in avocado seed. Meanwhile, narenginin was only detectable in kiwi fruit. From these results it could be noticed that the flavonoid compounds in avocado fruit and seed were more than kiwi fruit. Kosinska *et al.* [55] indicated that the avocado seed and peel are rich in flavonoid (quercitin). Pahua-Romos *et al.* [56] reported protocatechuic acid was the main phenolic compound in avocado seed powder flowed by kaempforide and vanillic acid.

Data in Table 6 showed that induced hypercholesterolemia caused a significant increase $P<0.05$ in body weight gain, feed intake and feed efficiency ratio (FER) in HC group G2 as compared with healthy control group G1. These results are in agreement with the findings of Lecumberri *et al.* [57] and Barakat and Lamiaa [58], they reported that rats fed high cholesterol diet showed significant increase in body weight gain. Administration of kiwi fruit and avocado (fruit and seed) to hypercholesterolemic rats caused a significant decrease in body weight gain and feed efficiency ratio at all levels except for diet supplemented 30% avocado fruit.

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Table 6: Changes of body weight, feed intake and FER in the hypercholesterolemic rats fed diet supplemented with kiwi fruit and avocado (fruit and seed).

Groups	*IBW (g) Mean ± SD	FBW § (g) Mean ± SD	*BWG (g) Mean ± SD	†FI (g/day) Mean ± SD	‡FER (g) Mean ± SD
G1 control(-)	115.5±2.83^{b}	166.3±2.28^{a}	50.8±2.00^{e}	13.00±0.84^{d}	0.093±0.25^{b,c}
G2 (HC) (+)	116.2±3.21^{b}	185.9±3.69^{a}	69.7±2.61^{a}	14.48±0.24^{a}	0.115±0.04^{a}
G3(HC)+10% Kiwi fruit	115.9±2.82^{b}	169±2.61^{de}	53.1±2.28^{bc}	13.98±0.53^{ab}	0.090±0.13^{c}
G4 (HC))+20% Kiwi fruit	117.1±1.41^{ab}	171.7±3.85^{cd}	54.6±2.28^{b}	14.32±0.26^{a}	0.091±0.08^{cd}
G5 (HC) +30% Kiwi fruit	117.4±1.41^{ab}	172.7±2.83^{bc}	55.3±3.69^{b}	14.4±0.22^{a}	0.091±0.05^{cd}
G6 (HC) +10% avocado fruit	119±1.26^{a}	175.6±3.16^{b}	54.6±2.61^{b}	14.17±0.42^{ab}	0.092±0.1^{bcd}
G7 (HC))+20% avocado fruit	116.8±2.28^{ab}	172.3±1.41^{bcd}	55.5±3.16^{b}	14.23±0.24^{a}	0.093±0.06^{b,c}
G8- (HC))+30% avocado fruit	117.9±1.41^{ab}	184.3±2.61^{a}	66.4±3.41^{a}	14.37±0.44^{a}	0.110±0.15^{a}
G9 (HC))+10% avocado seeds	118±1.41^{ab}	171.3±3.22^{cd}	53.3±3.69^{bc}	13.38±0.57^{cd}	0.095±0.18^{bc}
G10(HC))+20% avocado seeds	119±1.26^{a}	173.5±2.28^{bc}	54.5±2.28^{b}	13.63±0.4^{bc}	0.095±0.13^{bc}
G11(HC))+30% avocado seeds	117.8±1.41^{ab}	173.3±2.00^{bc}	55.2±2.28^{b}	13.93±0.42^{abc}	0.094±0.12^{bc}

*Initial body weight, § Final body weight, *Body weight gain, †Food intake, ‡Feed efficiency ratio
Mean with the same letters in the same horizontal column are not significantly different at $P<0.05$.

Table 7: Changes weight of organs in the hypercholesterolemic rats fed diet supplemented with kiwi fruit and avocado (fruit and seed).

Groups	Liver (g) Mean ± SD	Heart (g) Mean ± SD	Kidney (g) Mean ± SD	Spleen (g) Mean ± SD
G1 control(-)	6.1±0.67^{a}	0.500±0.08^{ab}	1.1250±0.19^{a}	0.325±0.09^{ab}
G2 (HC) (+)	7.12±0.91^{b}	0.550±0.05^{a}	1.25±0.17^{a}	0.325±0.05^{ab}
G3(HC)+10% Kiwi	6.42±0.92^{a}	0.475±0.05^{ab}	1.125±0.26^{a}	0.300±0.41^{ab}
G4 (HC))+20% Kiwi	6. 62±0.45^{a}	0.450±0.05^{ab}	0.975±0.15^{a}	0.275±0.05^{ab}
G5 (HC) +30% Kiwi	6.22±0.96^{a}	0.450±0.05^{ab}	1.125±0.45^{a}	0.350±0.01^{a}
G6 (HC) +10% avocado	5.72±0.76^{a}	0.450±0.05^{ab}	1.025±0.15^{a}	0.275±0.05^{ab}
G7 (HC))+20% avocado	6.47±0.71^{a}	0.450±0.129^{ab}	1.050±0. 05^{a}	0.300±0.1^{ab}
G8- (HC))+30% avocado	6.174±1.22^{a}	0.450±0.057^{ab}	1.00±0.18^{a}	0.300±0.1^{ab}

Table 7: Changes weight of organs in the hypercholesterolemic rats fed diet supplemented with kiwi fruit and avocado (fruit and seed).

Groups	Liver (g) Mean ± SD	Heart (g) Mean ± SD	Kidney (g) Mean ± SD	Spleen (g) Mean ± SD
G1 control(-)	6.1±0.67^{a}	0.500±0.08^{ab}	1.1250±0.19^{a}	0.325±0.09^{ab}
G2 (HC) (+)	7.12±0.91^{b}	0.550±0.05^{a}	1.25±0.17^{a}	0.325±0.05^{ab}
G3(HC)+10% Kiwi	6.42±0.92^{a}	0.475±0.05^{ab}	1.125±0.26^{a}	0.300±0.41^{ab}
G4 (HC))+20% Kiwi	6.62±0.45^{a}	0.450±0.05^{ab}	0.975±0.15^{a}	0.275±0.05^{ab}
G5 (HC) +30% Kiwi	6.22±0.96^{a}	0.450±0.05^{ab}	1.125±0.45^{a}	0.350±0.01^{a}
G6 (HC) +10% avocado	5.72±0.76^{a}	0.450±0.05^{ab}	1.025±0.15^{a}	0.275±0.05^{ab}
G7 (HC))+20% avocado	6.47±0.71^{a}	0.450±0.129^{ab}	1.050±0. 05^{a}	0.300±0.1^{ab}
G8- (HC))+30% avocado	6.17±1.22^{a}	0.450±0.057^{ab}	1.00±0.18^{a}	0.300±0.1^{ab}
G9 (HC))+10% avocado seeds	5.60±1.26^{a}	0.450±0.057^{ab}	1.025±0.15^{a}	0.275±0.05^{ab}
G10(HC))+20% avocado seeds	5.60±1.26^{a}	0.440±0.050^{ab}	1.025±0.16^{a}	0.275±0.05^{ab}
G11(HC))+30% avocado seeds	6.05±0. 085^{a}	0.475±0.050^{ab}	1.10±0.16^{a}	0.275±0.05^{ab}

Mean with the same letters in the same horizontal column are not significantly different at P<0.05.

Table 8: Effect of kiwi fruit and avocado (fruit and seed) on the serum lipid profiles of hypercholesterolemic rats.

Groups	*TG mg/dl Mean ± SD	°TC mg/dl Mean ± SD	†HDL-C mg/dl Mean ± SD	‡LDL-C mg/dl Mean ± SD
G1 control(-)	131.3±2.61^{k}	83.5±2.28^{k}	33.3±2.61^{a}	23.9±3.16^{f}
G2 (HC) (+)	273.2±2.83^{a}	137.0±3.69^{a}	23.3±3.16^{g}	59.1±2.28^{a}
G3(HC)+10% Kiwi fruit	219.1±2.83^{b}	122.6±3.41^{b}	27.3±2.99^{b}	51.5±2.98^{b}
G4 (HC))+20% Kiwi fruit	203.5±3.16^{c}	117.5±2.61^{c}	28.5±3.42^{b}	48.3±2.28^{b}
G5 (HC) +30% Kiwi fruit	195.9±3.22^{e}	117.6±3.35^{c}	29.7±2.61^{ab}	42.8±2.28^{c}
G6 (HC) +10% avocado fruit	212.3±2.83^{d}	119.9±3.41^{bc}	27.8±3.16^{b}	49.6±2.83^{b}
G7 (HC))+20% avocado fruit	199.2±2.61^{f}	113.6±2.28^{d}	29.3±2.18^{b}	44.5±2.98^{c}
G8- (HC))+30% avocado fruit	168.8±2.83^{i}	106.2±3.69^{e}	30.6±3.32^{ab}	41.8±2.00^{c}
G9 (HC))+10% avocado seeds	232.2±2.83^{b}	112.1±3.41^{d}	28.5±2.83^{b}	37.2±3.25^{d}
G10(HC))+20% avocado seeds	181.7±2.0^{h}	99.5±2.83^{f}	29.9±3.22^{ab}	33.3±3.31^{e}
G11(HC))+30% avocado seeds	155.2±2. 0^{j}	93.2±2.28^{g}	31.3±2.92^{ab}	30.9±2.83^{e}

*Triacylglycerol, °Total Cholesterol, †High density lipoprotein Cholesterol, ‡Low density lipoprotein Cholesterol
Mean with the same letters in the same horizontal column are not significantly different at P<0.05

Meanwhile, there are no significant difference was observed in feed intake of all groups. The reduced in body weight gain of kiwi and avocado fruit may be due to higher content of crude fiber (11.22% and 12.84%). The fiber reduced the gastric emptying rate and makes it possible for rats to feel full, which delaying the absorption and digestion of nutrients and reduced feed intake which lead to decrease body weight gain [59]. Our results are in agreement with Kim *et al.* [60], Shehata and Soltan [61], they reported hypercholesterolemic diet was supplemented with food rich in fiber (mulberry leaf powder, purslane and celery fresh) lead to decreases in body weight gain and the reduction of FER were depended on the levels of dietary fiber.

Effects of different concentration of kiwi fruit and avocado (fruit and seed) on weight of the main organs of hypercholesterolemic rat are shown in Table 7. Results revealed that liver weight was markedly increased in rats fed hypercholesterolemic diet compared to normal control group. Meanwhile, there are no changed in different other weight of organs. Supplemented diet of hypercholesterolemic rat with kiwi fruit and avocado

(fruit and seed) reduced liver weight compared to HC group. The lowering effect of avocado fruit and seed may be due to increases catabolism of lipid accumulated in adipose tissue causing a decrease in body weight. These results are in line with those obtained by Ojewele and Amabeark [62], who reported that the methanol extract of avocado leave provoked animal 8% decrease in mean liver weight compared to the hyperlipidemic control group.

The results of lipids profile which are presented in Table 8. Data clarified that hypercholesterolemic rats HC group recorded significant increase P<0.05 in TC, TG and LDL-c concentration as well as significant decrease in the level of good cholesterol HDL-c compared to healthy control group. These results are in agreement with Harnafi *et al.* [38] and Kumer *et al.* [63], they reported TC, TG and LDL-c showed significantly higher in hypercholesterolemic group than normal control group. Supplemented diet of hypercholesterolemic rats with different concentration of kiwi fruit and avocado (fruit and seed) lead to significantly decrease P<0.05 in TG, TC and LDL-c concentration as well as significant increase in level of good cholesterol HDL-c compared to HC group. The decrease in TC, TG and LDL-c level and increases in HDL-c were increases with increasing concentration of kiwi and avocado (fruit and seed). The present study indicated that the best results of lipid profile were observed of high concentration (30%) of kiwi fruit and avocado (fruit and seed). Feeding hypercholesterolemic rat on diet supplemented with 30% kiwi fruit reduced TC, TG and LDL-c by 14.16%, 28.29% and 27.58%, respectively and increased HDL-c by 27.46%. These results may be due to the micronutrient such as vitamin C (15.52%) and present phenolic compounds (258.55 mg/100d GAE), act as antioxidant to scavenge free radical and can delay or inhibit the oxidation of both lipid and other molecules [9]. These results are in line with These results are accordance with those reported by Mohammed [67], who revealed that the avocado fruit pulp administrated at doses 1 and 2ml/day/rat for ten week caused a significant decrease in the serum lipid including TC and TG levels and increased in HDL-c. Treatment hypercholesterolemic rat with 30% avocado seed reduced TC, TG and LDL-c by 31.97%, 43.19% and 47.72% respectively and increased HDL-c by 34.33% compared to HC group. The lowering effect of avocado seed of TC, TG and LDL-c may be due to the avocado seed had highest phenolic compound (285.43 mg/100g GAE) and flavonoid compounds (3.21mg/100g CE), soluble dietary fiber (38%) compared to kiwi and avocado fruit. These results agreement with Anderson [68] mentioned that dietary fiber, especially soluble, effectively decrease serum cholesterol and LDL-c concentration. Soluble fiber principle effects on cholesterol metabolism through decrease in bile acid absorption. These soluble fiber bind bile acids in the small intestine alter micelle formation and decrease their absorption, in the small intestine. Consequently, more bile acid is execrated with the feces. Flavonoid (Rosmarinic, Quercitrnic, Apignin and Hsperdine) and phenolic compounds (pyrogallol, syringic, catchin, procatechuic and catecal) which highly content in avocado seed acts as antioxidant protect of lipid peroxidation and scavenging to free radicals. Hung *et al.* [69] reported that flavonoid (quercetin, hesperidins' and namigin) have been shown to inhibit the generation or release of free radicals derived from lipoxygenase.

These results are in agreement with Asaolu *et al.* [70], who reported treatment hypercholesterolemic rat with various doses of methanolic of avocado seed (50. 100 and 200 ml) caused a significant reduction in the levels of TC, TG and LDL-c.

Data in Table 9 showed that the Levels of AI, LDL-C/HDL-C Ratio and HTR ratio of hypercholesterolemic rats administered diet supplemented

and other molecules [9]. These results are in line with those obtained by Maria *et al*. [64], who reported that diet containing kiwi fruit decrease of TG (61%), TC (29%) and LDL-c (38%). Chang and Liu [65] and Lim [66], they mentioned that regular consumption of kiwi fruit (Two kiwi fruit per day) might exert beneficial in hyperlipidemic subject.

Treatment hypercholesterolemic rat with 30% avocado fruit reduced TC, TG and LDL-c by 22.48%, 38.21% and 29.27%, respectively and increases HDL-c by 31.33% as compared to the HC group. These results may be due to avocado fruit are rich in monounsaturated fatty acid, fiber (12.84%), flavonoids (2.96 mg/100g CE), phenolic compound (259.15mg/100g GAE) and sterols. hypercholesterolemic rats administered diet supplemented with kiwi fruit, avocado fruit and avocado seed. Atherogenic index and LDL-C/HDL-C ratio increased significantly (4.9 and 2.5), while HTR% decreased significantly (17.0) in the HC g roup in comparison with the control group (1.5, 0.72 and 39.9), respectively. The atherogenic index and LDL-C/HDL-C ratio decreased according to the amount of kiwi fruit and avocado (fruit, seed) added in comparison with HC group and HTR% increased according to the amount of kiwi fruit and avocado (fruit, seed) added in comparison with HC group. The best level of kiwi fruit and avocado (fruit, seed) was 30% administration for hypercholesterolemic rats as compared to the HC group. Supplementation of the

Table 9: Levels of AI, LDL-C/HDL-C ratio and HTR of hypercholesterolemic rats fed diet supplemented with kiwi fruit and avocado (fruit and seed).

Groups	*AI Mean± SD	LDL/HDL Ratio Mean± SD	HTR Ratio Mean± SD
G1 control (-)	1.5±0.77^{c}	0.72±0.28^{c}	39.9±2.83^{a}
G2 (HC) (+)	4.9±1.77 ^{a}	2.5±1.14^{a}	17.0±2.83^{f}
G3(HC)+10% Kiwi fruit	3.5±1.58^{b}	1.9±0.85^{ab}	22.3±2.92^{e}
G4 (HC))+20% Kiwi fruit	3.1±0.79^{b}	1.7±0.46^{abc}	24.3±2.79^{e}
G5 (HC) +30% Kiwi fruit	3.00±1.14^{bc}	1.4±0.62^{b,c}	25.3±3.22^{de}
G6 (HC) +10% avocado fruit	3.3±1.04^{b}	1.8±0.77^{abc}	23.2±3.24^{e}
G7 (HC))+20% avocado fruit	2.9±1.36^{bc}	1.5±0.54^{b,c}	25.8±2.83^{cde}
G8- (HC))+30% avocado fruit	2.5±0.71^{bc}	1.4±0.51^{b,c}	28.8±2.83^{cde}
G9 (HC))+10% avocado seeds	2.9±1.49^{bc}	1.3±0.54^{b,c}	25.4±3.53^{de}
G10(HC))+20% avocado seeds	2.3±1.04^{bc}	1.1±0.46^{b,c}	30.1±2.91^{c}
G11(HC))+30% avocado seeds	2.0±0.85^{bc}	1.0±0.49^{cd}	33.6±2.28^{b}

*Atherogenic index
Mean with the same letters in the same horizontal column are not significantly different at P<0.05

Table 10: Activities of AST and ALT in serum of hypercholesterolemic rats fed diet supplemented with kiwi fruit, avocado (fruit and seed).

Groups	*AST U/mL Mean± SD.	°ALTU/mL Mean± SD
G1 control (-)	79.5±2.61^{g}	32.3±3.41^{g}
G2 (HC) (+)	123.5±2.28^{a}	56.5±2.61^{a}
G3(HC)+10% Kiwi fruit	113.6±3.16^{b}	47.9±3.41^{b}
G4 (HC))+20% Kiwi fruit	104.5±2.28^{c}	43.8±2.28^{c}
G5 (HC) +30% Kiwi fruit	92.0±3.22^{e}	39.5±3.41^{d}
G6 (HC) +10% avocado fruit	104.1±2.61^{c}	44.9±3.16^{c}
G7 (HC))+20% avocado fruit	97.6±2.83^{d}	39.4±2.61^{d}
G8- (HC))+30% avocado fruit	85.6±1.41^{f}	34.6±3.16^{e}
G9 (HC))+10% avocado seeds	110.8±2.28^{b}	44.4±2.28^{c}
G10(HC))+20% avocado seeds	95.5±3.22^{d}	38.5±2.0^{de}
G11(HC))+30% avocado seeds	82.2±2.83^{g}	33.8±1.79^{g}

*Aspartate aminotransferase, °Alanine aminotransferase.
Mean with the same letters in the same horizontal column are not significantly different at P<0.05.

Table 11: Levels of total cholesterol, triglyceride and GSH in liver tissue of hypercholesterolemic rats fed diet supplemented with kiwi fruit and avocado (fruit and seed).

Groups	*TG Mean ± SD mg/g	°TC Mean ± SD mg/g	†GSH Mean ± SD mg/g
G1 control (-)	12. 2±2.35^{d}	2.42±1.16^{d}	4.9±1.41 ^{a}
G2 (HC) (+)	18.9±3.35^{a}	3.8±1.25^{a}	3.0±1.14^{g}
G3(HC)+10% Kiwi fruit	17.1±3.31^{ab}	3.61±1.56^{ab}	3.69±1.43^{b}
G4 (HC))+20% Kiwi fruit	16.4±3.51^{abc}	3.41±2.25^{abc}	3.75±1.67^{b}
G5 (HC) +30% Kiwi fruit	15.0±2.83^{b-d}	3.2±1.52^{bc}	4.08±1.45^{b}
G6 (HC) +10% avocado fruit	16.7±3.41^{abc}	3.42±2.55^{abc}	3.84±1.42^{b}
G7 (HC))+20% avocado fruit	15.0±22.8^{b-d}	3.19±3.21^{bc}	4.17±2.02^{b}
G8- (HC))+30% avocado fruit	13.6±2.6^{b-e}	3.04±2.12^{bc}	4.33±2.2^{ab}
G9 (HC))+10% avocado seeds	15.0±3.22^{b-d}	3.34±1.92^{abc}	3.87±1.51^{b}
G10(HC))+20% avocado seeds	14.6±2.61^{b-d}	3.15±2.31^{bc}	4.11±1.44^{b}
G11(HC))+30% avocado seeds	12.8±1.88^{cde}	2.89±1.71^{cd}	4.4±1.76^{ab}

*Triglycerides, °Total Cholesterol, †Reduced Glutathione.
Mean with the same letters in the same horizontal column are not significantly different at P<0.05.

Cholesterol diet with 30% kiwi fruit and avocado (fruit, seed) improved the atherogenic index (AI) by about 38.8%, 49% and 59.2%, respectively. These results are in line with those obtained by Maria *et al*. [64], who reported that diets containing kiwi fruits significant decreased in the value of the atherogenic index by about 32%. These results also are accordance with the findings reported by Chang and Liu [65] and Lim [66], who indicated that after 8 weeks kiwi fruit consumption LDL-C/HDL-C ratio was significantly decreased in hyperlipidemic suffered subjects compared with hyperlipidemic subjects not consumed kiwi fruit. These results are in agreement with Maria *et al*. [71], who reported that treatment of mice with 125 mg avocado seed flour/kg BW significantly reduced the elevated level of AI by 50.6% compared to hypercholesterolemic mice. The atherogenic index markedly decreased causing a reduction in LDL-C/HDL-C ratio in all groups fed diet supplemented with kiwi fruit and avocado (fruit, seed). Our results are agree with Makni *et al*. [72], who stated that the increase in HDL-C or HTR ratio is one of the most important criteria of anti-hypercholesterolemic agent.

Effect supplemented diet of hypercholesterolemic rats with kiwi fruit and avocado (fruit and seed) on activities of AST and ALT are summarized in Table 10. Supplemented diet with different concentration of kiwi fruit and avocado (fruit and seed) reduced the AST and ALT activities compared to HC group. The best recorded of liver function was observed of higher concentration (30%) of all treatment. Supplemented died with 30% kiwi fruit and avocado (fruit and seed) reduced AST and ALT by (25.51% and 30.09%, 30.69% and 38.76%, 33.44 % and 40.18%) respectively. In this study, it is expected that the adding kiwi fruit and avocado (fruit and seed) to hypercholesterolemic diet well be effective for the recovery the hepatic function by improvement of lipid metabolism or delaying the hepatic disease. These results are in agreement with those reported by Mohammed [67], who indicated that rats which consumed 1 or 2 ml/day/rat avocado extract for 70 days showed decrease in AST and ALT activity compared to the control group. Also, Mohamed and Amr [73] reported that administration of dried avocado fruit at the three tests levels (5, 10 and 15%) caused lower of serum AST and ALT content compared to the control group.

Data in Table 11 illustrated that lipid level of liver tissue. High cholesterol diet caused significant increase p<0.05 of hepatic cholesterol (54.92%) and triacylglyceerol (57.02%) compared to the healthy control group. Administration hypercholesterolemic rat diet

supplemented with kiwi fruit and avocado (fruit and seed) at all levels reduced hepatic TC and TG. Meanwhile, the diet supplemented with avocado seed at all three concentration was the best to reduced hepatic lipid. Polyphenolics compounds of plants decrease the cholesterol level of liver tissue [74]. Liver cholesterol lowering effect may be due to avocado seed reduced absorption of cholesterol and fat or increases the fecal execration of fat and cholesterol [75]. The hypercholesterolemic rat's administration diet supplemented with 30% kiwi fruit and avocado (fruit and seed) had 20.6%, 28% and 32.2%, respectively, reduction in TG level in liver tissue. These results are in accordance with Imafidon and Amaectina [76], who reported that the hypertensive rats treated with 500 mg/kg avocado seed extract reduced TG by 36.19% compared to the control group.

Data in Table 11 also showed that the content of liver reduced glutathione (GSH) of hypercholesterolemic rats administered diet supplemented with kiwi fruit and avocado (fruit, seed). The content of GSH decreased significantly in the HC group in comparison with the normal group, while the groups fed the kiwi fruit and avocado (fruit, seed) GSH significantly increased with increases levels of kiwi fruit and avocado (fruit and seed) compared to HC group. Our results are in accordance with the data reported by Sadek *et al*. [77], who indicated that rats which consumed kiwi fruit caused a significant increase in glutathione content when compared to control rats.

CONCLUSIONS

The present study demonstrated that consumption kiwi fruit, avocado (fruit and seed) can modulate the risk factors of CVD (Cardiovascular diseases) by reducing the LDL-C, LDL-C/HDL-C and increasing HDL-C and HTR ratio. The results suggested that consumption of kiwifruit, avocado fruit and avocado seed might have some cardiovascular protective properties and beneficial effects on atherosclerosis, CVD risks in hypercholesterolemic rats.

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