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Analysis of Flavonoid Compounds in the Fruit of Prunus Microcarpa

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Abstract

The isolation and identification of flavonoid compounds, rutin and quercetin, in the fruit of prunus microcarpa (Rosaceae) are described. The flavonoid compounds have been extracted and separated by paper chromatography, thin - layer - chromatography, and flash coloum chromatography. Two compounds, rutin and quercetin, were identified by ^{1}H-NMR, IR, and UV. Rutin was hydrolyzed by 2M HCl /30 min., followed by extracting with ethyl acetate. Quercetin was obtained from the organic layer, and the aqueous layer was examined by paper chromatography and showed the presence of glucose and rhamnose.

The compound rutin(glycoside flavonoid) was quantified and the concentration found to be 57.081%.

Keywords:- ^{1}H-NMR,flavonoids, prunus microcarpa, T. L. C.

Introduction

Prunus microcarpa is a shrub of up to 0.5 - 3.5m. high from the Rosaceae family. Occurs wild in the kurdistan mountains and it is also can be seen frequent every where in the forest zone of kurdistan, occasional in the lower margin of the thorm - cushion zone[1]. A local name of prunus microcarpa in kurdish language is balalook, arabic name is (كمرز صغير الثمرة), and english name is little cherry. Nothing is known about its chemical composition, and literature survey indicates that no work has been reported with regard to the flavonoids on the fruit of prunus microcarpa.

Flavonoids are natural products which are universally, distributed in vascular plants including a large number of food plants.More than 2000 flavonoids are described[2], among them flavonols are the most important group. Most of these occur in plants in the from of glycosides. A large number of their glycosides have been reported in plants[2].

Flavonoids are yellow plant pigments that are widespread in nature. They belong to the benzopyran derivatives and are the most important natural pigments, together with the carotenoids and the tetra pyrole derivatives.

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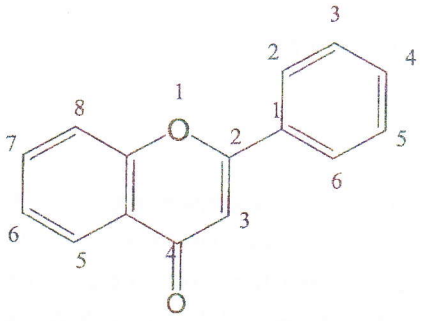
** Cited from her M. Sc. Thesis

Flavonoids have a typical chemical structure consisting of two benzene rings enclosing a hetro cyclic six membered ring containing an oxygen atom[3] (1).



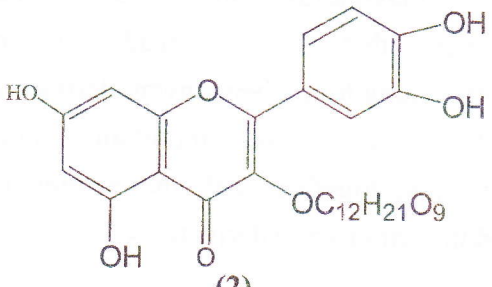
(1)

Typical chemical structures of the flavonoid and classification based on their heterocyclic ring.

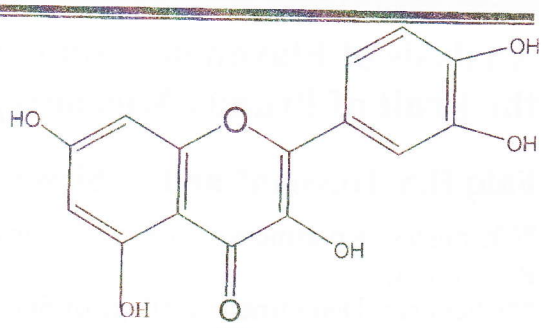
Rutin(glycoside flavonoid) is the 3-rhamno glycoside of 5,7,$\bar{3}$,$\bar{4}$ - tetra hydroxy flavonol(2) isolated first in 1842, from ruta graveolens.

It has been isolated subsequently from other 40 species of plants under many names[4].

Quercetin(3),a glycon of rutin which was isolated from the leaves of Eupatorium littorale[5].



(2)



(3)

It has been found that flavonoids have significant medicinal effects.Some flavonoids do support health as anti-inflammatory,anti-histaminic,and anti-viral agents[6],they also have immunomdulatory factor[7] moreover ,flavonoids have been found to have an over all lower risk of getting a wide variaty of cancer[8-9].

In fact ,quercetin has been found to inhibit both tumor promoters[10],and human cancer cells [11]on the other hand rutin has an important effect in resolving artevioscl erosis [12].It can reduce the edema of the low limbs of patient suffering from venous hypertension that it does so by promoting a reduction in capillary filteration[13].

In the present investigation, we describe for the first time isolation and identification of two flavonoids(rutin and quercetin) from prunus microcarpa.

Experimental Procedures

The fruit of prunus microcarpa have been collected in june 2000 in Sulaimani governerate(Iraqi Kurdistan). The fruit was allowed to dry in the

laboratory and ground through a wiley mill and stored tightly at 4C°. ^{1}H-NMR Spectroscopy: ^{1}H-NMR spectra obtained using a varian 60MHZ instruments with tetra methyl silane TMS as internal standard. I.R Spectroscopy: The infra-red spectra were obtained using Beijing second optical instruments factory, WQF - 300 FT IR spectrophotometer. UV spectroscopy: Ultraviolet data were obtained using PERKIN-ELMER HITACHI 200 spectrophotometer with recorder. T.L.C : standard silica gel(T.L.C) plates were used with 0.25 mm thickness.

Extraction of Flavonoids

In general alcohols are the solvent of choice for the extraction of flavonoids. The most useful solvent for all types of flavonoids from living tissue is 70- 80% methanol[14].

In this process, the dried powder fruit of prunus microcarpa was extracted with different solvents according to the scheme[15] (1).

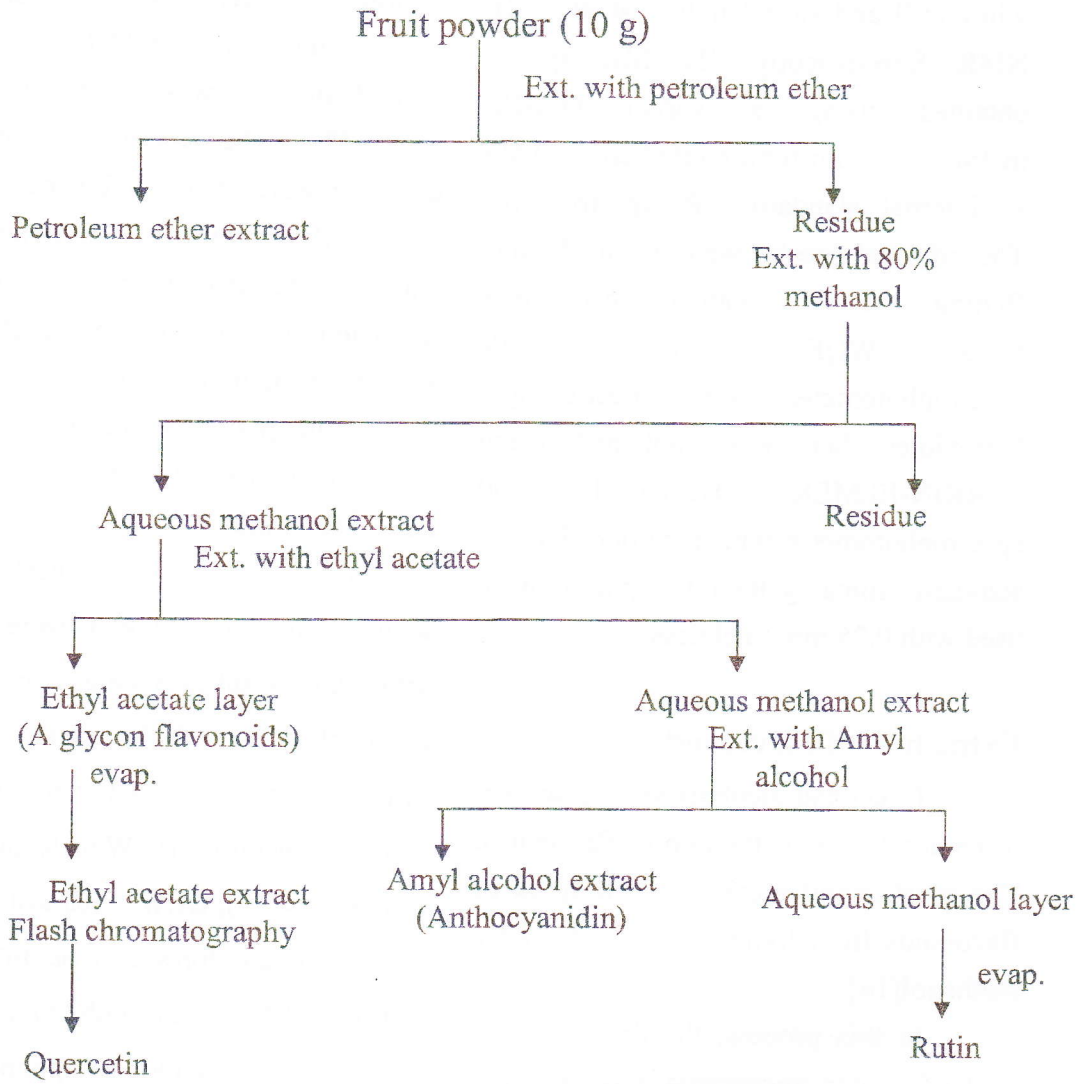
To examine the complexity of flavonoid mixture in ethyl acetate extract, and aqueous methanol layer in (scheme1) a simple two dimensional paper chromatography technique has been performed. The chromatogram was placed in an alcohol - acid mixture (BAW n-butanol : Acetic Acid : water 4:1:5 ahomogenous mixture(4:1:5) and the second direction was run in dilute acetic acid 15%). The solution of rutin, and quercetin were used as references.

Also thin-layer- chromatography was used to examin the complexity of flavoniod mixture in (ethyl acetate, and aqueous ,methanol layer).

The plates of silica gel of 0.25 mm. thickness were heated in an oven at 126 C° for 4 min.

The fraction of (ethyl acetate, and aqueous, methanol layer) were applied with capilary tubes a long a line (2 cm) above the rim of the plate. For this purpose the solvents used were 10% acetic acid in chloroform, BAW (n- bùtanol : Acetic Acid : water 4:1:5), BEW (n- butanol : Ethanol : water 4:1:5), and forestal (conc. HCl : Acetic Acid : water 3:30: 10) as amobile phase.

The flavoniod compounds were detected by spraying with (1% $AlCl_3$ in 5% Aqueous ethanol) and under UV. light before and after fuming with the vapour from a jar of concentrated ammonia.Solution ofrutin and querctin have been Solution of rutin, and querctin have been used as authentic markers[16].



Flash Chromatography

The column (5×25cm.) poured with cellulose has been used for separation of flavonid compounds in (ethyl acetate extract, and aqueous methanol layer). Preparatively using 30 to 100% methanol to display different types of flavoniods[16].

Table (1): R_f Values of Rutin, and Quercetin in paper Chromatography

Flavonoid	$R_f \times 100$	
	BAW	15% HOAC
Rutin	43	50
Quercetin	64	3

Table (2): R_f Values of Rutin, and Quercetin in Thin- Layer – Chromatography

Flavonoid	$R_f \times 100$			
	10% HAC in $CHCl_3$	BAW	BEW	Forestal
Rutin	50	51	50	34
Quercetin	66	84	89	42

Hydrolysis of Glycoside flavoniods (Rutin)

1. The amount of the aqueous methanol extract which is corresponding to glycoside flavonoids (rutin) was hydrolyzed with 2 M HCl for 30 min. This aqous layer was extracted with ethyl ecetate by using separation funnel and quercetin which was obtained from hydrolysis of rutin was identified in this layer by chromatographic comparision with an authentic solution of quercetin.

2. For removing acid (HCl) from the aqueous residue an ion exchanger (Ambertlite) IR-45 (OH) BDH chemicals Ltd.. was used and the sugars which were proposed to be glucose and rhamnose were received from column, and identified by[16]:-

Paper Chromatography:-

The sugar solution which was received from the column was run one dimensionally by ascending chromatography in BAW and BEW on whitman's No. 1 (7×10 cm) paper with a standard solution of both glucose and rhamnose as authentic markers[16].Good separation of rhamnose and glucose were achieved with 19 hrs run. The dried paper was then dipped in aniline hydrogen phthalate, and then dried.

Finally, the paper was heated at 105 °C for 5 min in order to develop the distinctive colours[15].

Table (3): R_f Values, Colour and Solvents for Paper Chromatography of Glucose and Rhamnose

Sugar	Rf (× 100) in		Colour with Aniline Hydrogen Phthalate
	BAW	BEW	
Glucose	20	13	Brown
Rhamnose	61	62	Pale brown

Solvent key

BAW: n- butanol : Acetic Acid : water 4:1:5

BEW: n- butanol : Ethanol : water 4:1:5

Forestal: conc. HCl : Acetic Acid : water 3:30: 10

Results & Discussion

The flavoniod compounds (glycoside and a glycon flavonoids) were separated from prunus microcarpa fruit by extraction of the defatted fruit powder with methyl alcohol and then by ethyl acetate as shown in scheme 1.

Simple two dimensional paper chromatography and one dimensional T. L. C examined the complexity of the ethyl acetate extract, and aqueous methanol layer respectively (scheme 1).

The paper and thin- layer chromatography for ethyl acetate extract showed two spots, first spot which was corresponding to quercetin was separated by flash column chromatography and identified by $_1$H-NMR , IR. , and UV. spectroscopy.

The second spot corresponding to rutin was separated and identified from aqueous methanol layer.

band at 1665 cm^{-1} could be attributed to carbonyl group, bands at 1640 cm^{-1} and (1575-1477) cm^{-1} indicate the existence of

IR- spectrum table(4) of the quercetin showed a broad band at (3600-3200)cm^{-1} corresponding to hydroxyl group (bonded). The second

C-O group. The bands below 900 cm^{-1} for C-H out of plane indicate the presence of aromatic protons.

The $_1$H-NMR spectrum table(5) of quercetin showed a broad signal at δ (2.5-5.5ppm)for m-OH of OH groups and the multiplet signal at δ (7-8)ppm corresponding to five protons of two phenyl groups. The ultraviolet spectrum of quercetin showed absorption bands at 234, 269, and 303 nm.

Table (4): IR Spectral Data of the Compounds Quercetin and Rutin

compounds	cm^{-1}	Signal	Assignment
Quercetin	3600-3200	Broad band	O-H group bonded
	1665	Signal band	C=O group
	1640	Signal band	C=C group of an aromatic ring
	1575-1477	Multiplet	C=C group of an aromatic ring
	1300-1000	Multiplet	C-O group
	below 900	Multiplet	C-H bending of aromatic protons
Rutin	3600-3000	Broad band	O-H group bonded
	2900	Signal band	CH_2, CH_3 aliphatic
	1715	Signal band	C=O group
	1638-1484	Multiplet	C=C group of an aromatic ring
	1433-1368	Multiplet	CH_2, CH_3 bending
	1300-1000	Multiplet	C-O group
	below 900	Multiplet	C-H deformation of an aromatic proton
	829	Distinct band	α-D-glycoside linkage

Identification of flavoniod glycoside (Rutin) has been done by paper, thin-layer chromatography, and spectral measurments IR, $_1$H-NMR , UV.

The IR spectrum table (4) of the compound rutin showed the broad band (3600-3000)cm-$_1$ corresponds to hydroxyl group (bonded) ;the band at 2900 cm^{-1} could be attributed to aliphatic CH_3 and

CH_2 groups the band at 1715 cm^{-1} indicates the presence of carbonyl group. The bands at (1638-1484) cm^{-1} may be attributed to C=C group of an aromatic ring, bands at (1433-1368) cm^{-1} due to the CH_3 and CH_2 , bending, and the present bands at (1300-1000) cm^{-1} indicated C-O group, while the bands below 900 cm^{-1} indicated the existence of C-H deformation of an aromatic proton. Finally a distinct band at 829 cm^{-1} indicated the presence of α-D-glycoside linkage[16].

The ^{1}H-NMR spectrum table(5) of the compound rutin showed a multiplet signal at δ(1.7-2.3)ppm for $CH_2$and CH_3 protons. The broad signal at δ (2.4-5.0)ppm corresponding to protons of OH groups and finally the multiplet signal at δ (7-8)ppm corresponds to protons of the two phenyl groups.

The ultraviolet spectrum of rutin showed absorption band at 233, and 266nm. The compound rutin (flavoniod glycoside) was hydrolyzed with 2 MHCl for 30min. , extracted with ethyl acetate, and quercetin has been identified in this layer by chromatgraphic comparison with an authentic sample of quercetin. The aqueous residue has been passed through ion exchange resin for removing the mineral acid, which was used for hydrolysis. The sugars which were obtained from hydrolysis of the compound rutin were identified by paper chromatography with authentic sugar markers (glucose and rhamnose) they have same Rf values table (3).

It is also important to note that the percentage of rutin is higher in the flesh of prunus microcapa fruit (57.081%).

Table (5): ^{1}H. N. M. R. Spectral Data of the Compounds Quercetin and Rutin with TMS as internal standard , 60 MHZ, D_2O .

compounds	δ in ppm	Signal	Assignment
Quercetin	2.5-5.5	5 H broad	OH groups
	7-8	5 H Multiplet	Two phenyl groups
Rutin	1.7-2.3	Multiplet signal	CH_2, CH_3 protons
	2.4-5.0	Broad signal	OH groups
	7-8	Multiplet signal	5H for two phenyl groups

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شیکردنەوەی ئاوێتە فلافۆنۆیدەکان لەناو میوەی بەڵاڵوک (*Prunus microcarpa*)

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پوختە

لەم توێژینەوەیەدا توانرا ماددە فلافونویدەکان دەربهێنرێت (Flavonoids) لەناو میوەی داری بەڵاڵوک (Prunus microcarpa) وە جیابکرێتەوە بەڕێگەی کرۆماتۆگرافیای کاغەز (P.C) یان بەچینی تەنک (T. L. C.)وە هەروەها بە هۆی کرۆماتۆگرافیای فلاش (Flash Chromatography). هەردوو ئاوێتەی روتین لەگەڵ کویرستین دیاری کران بەڕێگەی (^{1}H-NMR , IR, UV) روتین توانرا شیبکرێتەوە بە هۆی (2MHCl) بۆ ماوەی (30 min.) (Reflex) وە دوای دەرهێنانی بەتوێنەری ئیسایل ئەسیتەین توانرا کویرستین لە چینی ئۆرگانیک دا جیابکرێتەوە وە چینی ئاوی توانرا جیابکرێتەوە بەڕێگەی کرۆماتۆگرافیای کاغەز وە شەکری کلوکوز لەگەڵ رامنۆز دیاریکران.

وە هەروەها لەم توێژینەوەیەدا دەرکەوت کە ڕێژەیەکی زۆر لە ماددەی روتین (گلایکۆساید فلافونوید) لەناو میوەی بەڵاڵوک (Prunus microcarpa fruit) دا هەیە وەڕێژەکە بریتی بوو لە 57.081% .

تحليل مركبات الفلافونويدات في ثمرة نبات (*Prunus microcarpa*)

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الخلاصة

في هذ البحث تم استخلاص و فصل المركبات فلافونويدات (Flavonoids) في ثمرة نبات (prunus microcarpa) باستخدام الكروموتوغرافيا الورقي (P.C) ، كروموتوغرافيا طبقة الرقيقة (T. L. C.) و كروموتوغرافيا فلاش (Flash Chromatography) . و تم تشخيص مركبين روتين (Rutin) و كويرستين (Quercetin) باستخدام (^{1}H. N. M. R. , IR, UV) . المركب روتين تحلل باستخدام (2MHCl) ثم استخلص بواسطة مذيب (Ethyl acetate) خلات اثيل، اما مركب (Quercetin) كويرستين فظهر في الطبقة العضوية، و الطبقة المائية شخصت باستخدام كروموتوغرافيا الورقي(P.C) ظهر بانه كلوكوز و رامنوز و ظهر في النتائج ان نسبة مركب روتين (Rutin) في ثمرة نبات (Prunus microcarpa) كثيرة و نسبة 57.081% .

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وەرگیرا لە ٢٠٠٢/٧/٢٧ وپەسەندکرا لە٢١/٤/ ٢٠٠٣ز.