Sudan University for science and Technology

College of Science

Department of Scientific Laboratories –Chemistry

Determination of Minerals content and Flavonoids of pomegranate peels

تقدير المحتوى المعدني والفلافونيدات في قشور الرمان

A dissertation submitted in partial fulfillment for the degree of B.sc(Honor)

in scientific laboratories- Chemistry

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بسم الله الرحمن الرحيم

قال تعالى

﴿ وَهُوَ ٱلَّذِىٓ أَنزَلَ مِنَ ٱلسَّمَآءِ مَآءً فَأَخۡرَجۡنَا بِهِۦ نَبَاتَ كُلِّ شَىۡءٖ فَأَخۡرَجۡنَا مِنۡهُ خَضِرٗا نُّخۡرِجُ مِنۡهُ حَبّٗا مُّتَرَاكِبٗا وَمِنَ ٱلنَّخۡلِ مِن طَلۡعِهَا قِنۡوَانٞ دَانِيَةٞ وَجَنَّٰتٖ مِّنۡ أَعۡنَابٖ وَٱلزَّيۡتُونَ وَٱلرُّمَّانَ مُشۡتَبِهٗا وَغَيۡرَ مُتَشَٰبِهٍۗ ٱنظُرُوٓاْ إِلَىٰ ثَمَرِهِۦٓ إِذَآ أَثۡمَرَ وَيَنۡعِهِۦٓۚ إِنَّ فِي ذَٰلِكُمۡ لَأٓيَٰتٖ لِّقَوۡمٖ يُؤۡمِنُونَ ﴾صدق الله العظيم (سورة الانعام) – الايه 99

Dedication

I dedicate this work to my parents, who gave me the necessary and valuable guidance that lead me to where I am standing today …….

To my brothers,sisters ,friends and colleagues…

Acknowledgment

Thanks to Allah almightly for his blessings. we would like here to thank our supervisor:

Dr/ Omer Adam Gibla for his help , guidance and encouragement.

Our thank would extent to ustaz Ameen , Ahmed and Abd EL-Hameed .

Lastly , we would like to thank our friends for friendship through all these years…

Abstract

The aim of this study is to isolate the flavonoids from pomegranate peels in ethanolic extract , and identify the different types of flavonoids . The study also aimed to determine the minerals content in the pomegranate fruit .

Flame photometer, U.V/Vis. spectrophotometry, X-rays fluorescence and Infrared techniques were used.

The results of analysis showed that, there is a high concentration of potassium, calcium and Iron in the seeds. Also showed a low concentrations of lead, chromium, manganese, copper , zinc , rubudium , bromine and stranchium .

The results showed that pomegranate peels contain flavonoids and alkaloids .

According to the results of U.V/Vis and IRthe type of flavonoid was flavonol .

ملخـــص البحـث

تهدف هذه الدراسة الى عزل الفلافونيدات من قشور الرمان في مستخلص ايثانولي وتصنيفها, وتقدير المحتوى المعدني في قشور الرمان.

أستخدمت تقنيات مطيافيه اللهب,الطيف الفوق البنفسجي المرئي ,مطيافيه الاشعه تحت الحمراء وفلوره الاشعه السينيه.

أظهرت نتائج الدراسة وجود تراكيز عالية من معادن البوتاسيوم,الكالسيوم والحديد, وتراكيز منخفضه من الرصاص,الكروم,المنجنيز,الزنك,البروم,الاسترانشيوم والروبيديوم .

أظهرت النتائج احتواء قشور الرمان على الفلافونويدات والالكولويدات , وعدم احتوائها على السكريات , ووجد أن نوع الفلافونويد الموجود في القشور هو الفلافونول .

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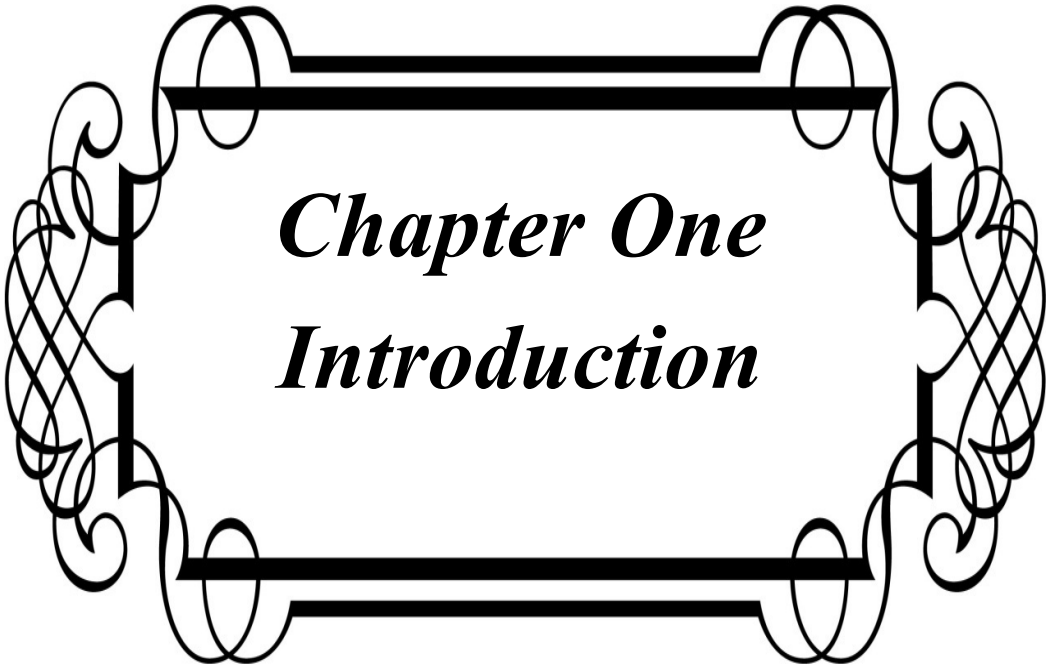

Chapter One
Introduction

1-Pomegranate

1-1Classification of pomegranate

The pomegranate (punicagranatum .L) is an ancient fruit it has been widely consumed in various cultures for thousand of years .The use of pomegranate fruit dates back to biblical times and reports of its therapeutic qualities have echoed throughout the millennia the Babylonian regarded pomegranate seeds as an agent of resurrection the Persians believed the seeds conferred invincibility on the battle fields, while for the ancient chines the seeds symbolized longevity and immortality .

Order: Myrtales

Family: Punicace

Gender: punica

Specie: punicagranatum

1-2Botanic Description

1-2-1 Punicagranatum is a small multi_ stemmed shrub /tree 5-10 tall. Canopy open, crown base low Stem woody and spiny, bark smooth and dark grey.

Leaves: simple, 2-8 cm long, oblong or obovate , glabrous, oppositely placed, short petioled surface shining.

Flowers regular, solitary or in fascicles at apices,4-6 cm petals lanceolate 5-7,wrinkled and brilliant orange _red. Hypanthium colored, 5-8 lobed anthers numerouscalyx persistent.

Fruit around berry,5-12cm,pericarp leathery .Interior compartmentalized with many pink _red sections of pulp _cike tissue .each contains a seed grain .fruits globose with persistent callipe and a coriaceous woody rind.

Seeds numerous, angular with fleshy test, 1.3 long

1-2-2 Tow subspecies are recognized on base of ovary color,subspchlorocarpa and prophyrocapa .numerous cultivars ,some dating to the 13 century , are known .

The specific epithet granatum and derives from latingraum(grain) and means "many-grained".

Only two species ,p.granatum and p.protopunica , are known for this monogenric family with close affiliation to the lythraceae . p.protopunica is endemic to Socotra and is listed as an endangered plant in the IUCN red list .[1]

1-3Biology

This is a herm a phroclitic species .flowering is observed from mid _April to may in India .fruiting begins in the 7^{th} or 8^{th} year and fruits take 6-7 months to mature.

The number of fruits may vary from 20-25 for young trees to100-150 for 10 years old trees and even 200-250 for older trees yield varies with tree size. [1]

1-4 Ecology

Pomegranate is susceptible to fire, first at -11deg c damage to tree is irrecoverable tolerates soil compaction,drought and seasonal water logging.

Biophysical limits:

Altitude: up to 150m

Mean annual temperature: 20 deg C

Soil type: prefers well drained, heavy, light and medium soils .will also do will calcareoussoils.

1-5Documented Species Distribution

Native: Afghanistan, Iran, Libyan Arab Jamahiriya.

Exotic : Egypt ,Greece , India , Indonesia , Israel ,Italy ,Kenya ,Marco ,Pakistan ,Saudi Arabia , Spain ,Sri Lanka, Tanzania ,Turkey , Russian federation and USA.[1]

1-6Products

1-6-1Food: The seeds have a fresh, sweet – sour, very pleasant taste.

1-6-2Fodder: The leaves are browsed by domesticated stock.

1-6-3Fuel: Tree branches used for fire wood.

1-6-4Tannin or Dyestuff: The root bark yield ablack ink rich in tannin and useful in dyeing tannin leather.

1-6-5Medicine :The bark of the pomegranate tree may be used as a very strong purgative ,but it has several serious –side effects .The fresh root bark is used in anthelmintic preparation ,the alkaloid punicine is responsible for this activity .unripe fruit and flowers are significant emetics .Rip fruits are laxative and blood enriching also useful in managing sore throat , eye ,brain disses and chest troubles .[1]

The pomegranate,punicagranatum is also world fruit originating in the middle east and even usually grow to about 6m tall and remain productive for many years .The plants may be grown as amultiple –stemmed shrub or a signal –stemmed tree .The latter is preferred.

1-7Climate and Soil

The pomegranate plant is very adaptable and will grow in regions ranging from temperature to tropical .It is deciduous or some deciduous depending on its location .The best prospects for commercial fruit production is where the summer is warm to hot and where the rain fall is minimal during late summer – autumn .Water should be available .For irrigation ,if water is kept up to the plant the chances of rain fall splitting the fruit will

be reduced .Rain fall , however can cause soft fruit and bring in undesirable diseases . Very hot weather can lead to sunburn in jury on fruit.

Deep,loamy,well –drained soils are preferred but the pomegranate has same tolerance to less than ideal drainage and to mild alkaline condition.[2]

1-8 Irrigation and Nutrition

Although the pomegranate tree is very drought –tolerant it needs adequate moisture to product good crops .Adequate soil moisture as fruit approaches maturity is said to reduce the susceptibility of the fruit to splitting .Overseas experience indicates that fully grown trees will benefit from one or more applications of fertilizer which is total provide 0.25 to 0.5 kg nitrogen annually. [2]

1-9 Harvesting, Packing and Storage

Fruiting should commence by the trees third to fifth year age if there is a large amount of fruit to be handled her vesting can begin a little before full maturity because ripening will proceed airing the postharvest and storage periods .If harvesting is delayed the chance of fruit loss as a result of splitting will increase. The pomegranate is thick and storage the fruit is best removed by chipping. Remove only fruit that is adequately size and colored at the first pick.[2]

1-10 Chemical Composition of Pomegranate

The pomegranate fruit has valuable compounds in different parts of the fruit. These can be divided into several anatomical origins, peel,seed and arils.

The chemical composition of fruit differs depending on the cultivar growing region, climate,maturity, cultivation practice and storage condition.[3][4]

Principal constituents of different parts of pomegranate and fruits.

Significant variation in organic acid , phenolic compounds, sugars, water –soluble vitamins and minerals of pomegranate have been reported over the years by various researches [5].About 50%of total fruit weight corresponds to the peels which is important source of bioactive compounds such as phenolics,flavonoids and ellagitannins [6].

1-11 Constituent

Many efforts have been made during the last decades to investigate the constituents of pomegranate, leading to the isolation and structure characterization of many compounds which are categorized into poly phenols including tannins and flavonoids, alkaloids, organic acids.

1-11-1 Tannins

Hydrolysable tannins of diverse structures including ellagitanins and galoo tannins constitute the most prevalent compounds withhowever are rarely found in this plant.

Ellogi tannins are mainly found in the pericarp, bark, seeds and flowers [7].These compound, synthesized through esterification, lactonization and glycosidation between the moieties for single to multiple molecules.

1-11-2 Alkaloids

Were mainly found in the bark of both stem and root as well as the juice of pomegranate. They are mainly two kinds of alkaloids including piperidinses and pyrrolidines reported in plant.

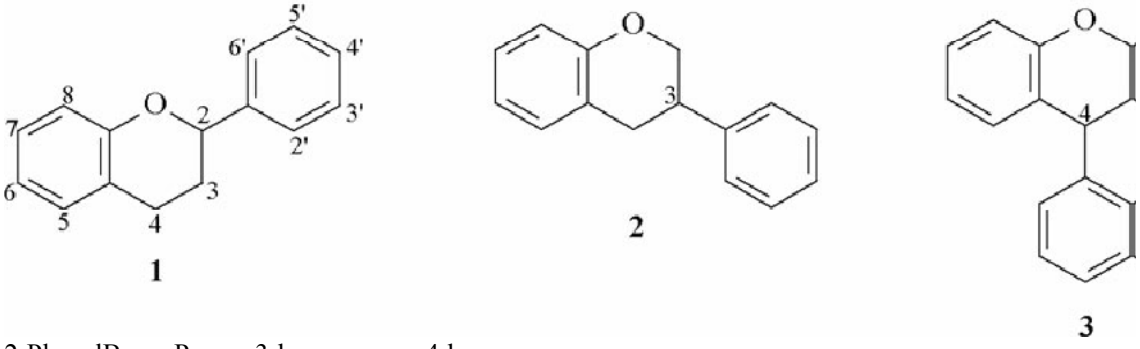
1-11-3Terpenes and Steroids

Triterpenes with glycosidation are frequently found in the flower and seeds of pomegranate. These compounds usually appear in the form of pentacyclic tri terpenoidswith a c-28 carboxyl and a double bond between c-12 and c-13.

1-11-4Flavonoid

Flavonoids isolated from pomegranate include flavonol , flavonois ,anthocyanidins and flaven -3-ols .The brilliant colors of pericarp and juice are attributed to anthocyanidins and flavan-

3-ols , of which the content decrease or increase with the time of ripening. Flavones and flavonols constitute the major flavonoids of pericarp leaves. The term "flavonoid" is generally used to describe abroad collection products that include a C6-C3-C6 carbon frame work this group of natural products may be divided into three classes: Flavonoids 1, ISO Flavonoids2, NEO Flavonoids.[8]



2-PhenylBenzoPyrans 3-benzopyrans 4-benzopyeans

Fig1-11-4-1 : flavonoids classification

Based on the degrees of oxidation and saturation present in the hetero cycle c-ring the flavonoids may be divided into the following groups:

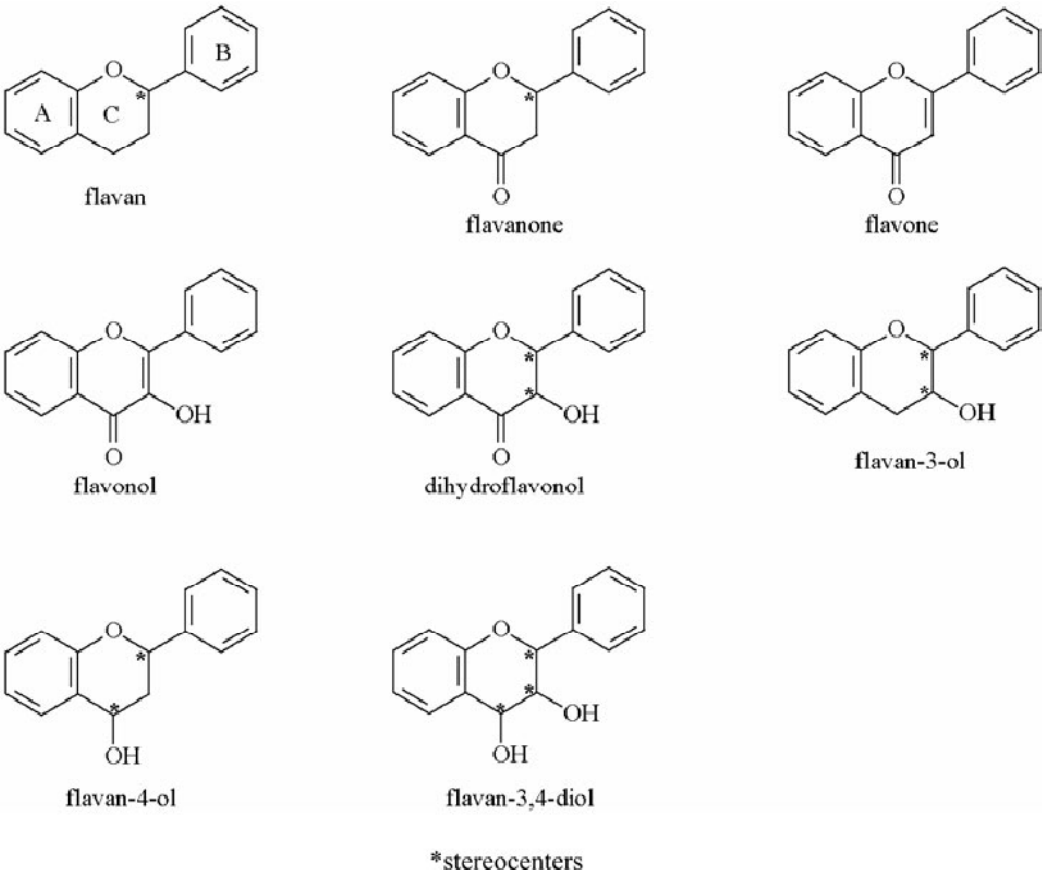


Fig 1-11-4-2 : flavonoids groups

1-12Classification of Flavonoids

1-12-1Flavones

They have a double bond between positions 2and3 and a ketone in position 4 of C ring. Most flavoneshavea hydroxyl group in position 5 of the A ring.

1-12-2Flavonols

Compared to flavones, they have a hydroxyl group in position 3 of the C ring, which may also be glycosylated.

1-12-3Flavonones

Also called dihydroflavones , have the C ring saturated , therefore unlike flavones , the double bond between position 2 and 3 is saturated and this is the only structural difference between the subgroups of flavonoids.

1-12-4Flavanonols

Also called dihydroflavonols, are the 3-hydroxy derivatives of flavanones, they are an highly diversified and multi substituted sup group.

1-12-5Isoflavones

An anticipated, isoflavones are a sub group of flavonoids in which the B ring is attached to position 3of the C ring they have structural similarities to estrogen such as estradiol, and for this reason they are also called phytoestrogens.

1-12-6Neoflavonoid

They have the B ring attached to position 4 of the c ring.

1-12-7Flavanols or Flavan-3-ols or Catechins

The hydroxyl group is almost always bound to position 3 of C ring , they are called catechins as well.

1-13Bioactivties

Different parts of the plant have multiple bioactivties such as hoypolipidemic, antiviral, antioxidant, anti-neoplastic, antibactecterial, anti-diabetic, anti-diarrheal and helminthic effects.

1-13-1 Anti-pathogenic microbes

1-13-1-1Anti-bacteria

Besides the helicobacter pylori, Escherichia coli, salomnellatyphi and microorganisms of shigella the extracts of pomegranate also exhibit significant in habiting effect against the common pathogenic bacteria especially gram positive pathogens it was reported that both methicillin-resistant (MRSA)and methicillin sensitive (MSSA) strains of staphylococcus aureus were susceptible to the extracts of pericarpor fruit and the subsequent enterotoxin production was inhibited by these extracts.[9]

1-13-1-2Antivirus

Polyphenols especially tannins of pomegranate play a key role in the antiviral effect because of their special property of protein precipitation which adversely effect the enzymes involved in the life cycle of virus [10].

1-13-2 Anti-angiogenesis

Ti is important for the tumor growth and metastasis that new blood vessels can regenerate and develop timely to supply oxygen and nutrients in the tumor cells. The juice and the seeds soil of pomegranate could adversely affect the angiogenesis by down regulating the pro-angiogenic vascular endothelial growth factor (VEGF)in MCF-7 estrogen dependent breast cancer cells[11].

1-14 Relative studies

1-18-1 The study on the proximate composition, minerals content antibacterial and antifungal activities of the most popular consumed fruit pomegranate has been carried out using recommended method of analysis.(Sarhad university of science and information technology, Pakistan 2012).

1-18-2The effect of two method of pomegranatejuce extraction on quality during storage at 4c. (biomedbiotchnol dec.2004).

1-18-3 The Ant microbial activity of five plant extracts (spearment) (butnij) (colocynth) (bambar) (pomegranate) on various isolates of bacteria, fungi and yeasts.(Basra university 2002).

1-18-4 Antioxidant, antimalarial and antimicrobial activities of tannin-rich fraction, Ellagitannins and phenolic acids from

punicagranatum.L (Department of pharmacognosy, school of pharmacy ,university of Mississippi 2007).

Chapter Two

Materials and Method

2-Materials and Method

2-1 collection of samples

Pomegranate fruits collected from local market of Omdurman town.

2-2 Chemicals

2-2-1 Hydrochloric acid pure, Assay (35-38%)

Lobachemiepvt.ltd.India

2-2-2 Petroleum ether, B.P (60-80) c

2-2-3 Acetic acid, glacial, Assay 99.5%

Alpha chemika,India

2-2-4 Methanol,Assay 99%

2-2-5 Sulphuric acid, Assay 98%

Oxford lab chem

2-2-6 Iodine, Assay 98.5%

2-2-7Ethanol, Density 0.789 at 20c, Assay 95%

2-2-8 N-butanol, Density 0.8120 at 20c, Assay 99%

2-2-9 Aluminum chloride, M.P 192.4c, Assay 99%

2-2-10 Boric acid, M.P 170.9c, Assay 99%

2-2-11 Potassium Iodide, M.P 304c, Assay 98%

LobaChemia,India

2-2-12 Bismuth Nitrate, Assay 99%

2-2-13 Silica gel

Techno pharm chem, India

2-2-14 Sodium acetate, Assay 99%

Alpha chemika,India

2-2-15 Sodium Metal, Assay 99%, M.P 97c

Central drug house, India

2-2-16 Phenaline

2-3Instruments

2-3-1 U.V /Vis Spectrophotometer

Company: JENWAY

Model:6505 UV/Vis spectrophotometer

2-3-2 Fourier Transform Infrared (FTIR)

8200- JENWAY

2-3-3 X-ray fluorescence (XRF)

2-3-4 Flame photometer

Company: JENWAY

Model pfp7 flame photometer

2-4 Methods of analysis

2-4-1 Preparation of sample

After collection of sample, we let dry at room temperature,then stored away from sun lights and ,then stored at proper conditions.

2-4-2 Extraction procedure

5oog of peels were added to a clean dry beaker then 1500ml of ethanol were added. The beaker content shacked rapidly and left for 72 hour. The extract was evaporated at room temperature for two days, them filtered and store in a brown glass bottle.

2-4-3 Selection of solvent

The appropriate solvent system to separate the flavonoids from the extract had been chosen from different solvent systems according to TLC separation results.

A mixture of butanol, acetic acid and water (4 : 1 : 5) , and mixture of acetic acid and water .

The appropriate solvent was water and acetic acid (8:2).

2-4-4TLC Separation

Silica gel had been dissolved in distilled water, then we made a thin layer of it , let it dry, a few drops of the extract were dropped at it in a shape of line , put it in a solvent system of acetic acid and water (2:8).

2-5 Identification Tests

2-5-1 Flavonoids Test

2-5-1-1 Aluminum chloride Test

1g of aluminum chloride had been dissolve in 100 ml of methanolto 3ml of extract a few drops of solution were added.

2-5-1-2 Potassium hydroxide Test

1g of potassium hydroxide was dissolved in 100ml of DW to 3ml of the extract few drops of potassium hydroxide were added.

2-5-2 Alkaloids Test

2-5-2-1 Wagner reagent

5g of Iodine and 10g of potassium iodide was dissolved in 100ml distilled water ,5ml of (2N)hydro chloric acid was added to cool crude solution few drops of Wagner reagent was added.

2-5-2-2 Mayer reagent

1.5g of Mercury chloride and 5g of potassium iodide was dissolve in 100ml of water the solution was added to the extract.

2-5-3 Glycosides Test

20ml of extract was shacked in test tube.

2-6 Infrared Spectrophotometric Analysis

After pressing the sample with potassium bromide salt with percentage (1: 10), then we put it in the path of IR radiation.

2-7 U.V/Vis Spectrophotometric Analysis

Flavonoids sample was prepared, by dissolving a mount in methanol. After filling the cell of the spectrophotometer with stock solution ,reading were obtained ,then we filled the cell with sodium meth oxide solution ,then we filled the cell with the sample adding to sodium acetate salt, then we disposed the content and filled the cell with sample and drops of aluminum chloride , after that we ,reading were obtained ,then we filled the cell with sample solution and drops ofboric acid salt after dissolved it in methanol ,then reading were obtained.

2-8 XRF Analysis

One gram of pomegranate seeds powder was introduced to XRF instrument, the instrument was operated and concentration of some minerals was determined

2-9 Flame Photometer Analysis

0.5g of pomegranate ash was dissolved in HCL (2M) and transferred to 50ml volumetric flask and the volume was completed to the mark with distilled water.

Flame photometer was used for determination of potassium ion.

Chapter Three

Results and Discussion

3-Result and Discussion

3-1Qualititaves Test Result

3-1-1Flavonoids Test

the results showed that the pomegranate peels contain flavonoids.

Table 3-1 : Flavonoids test results

Test	*Result*
Aluminum chloride (III)	Positive "dark brown color"
Potassium hydroxide	Positive "dark yellow color"

3-1-2Alkloids Test

the results showed that pomegranate peels contain alkaloids.

Table3-2 : Alkaloids test results

Test	*Result*
Wagner	Positive "precipitate"
Mayer	Positive "precipitate"

3-2Infra red Results

The sample of flavonoids show multiple peaks in the spectrum of IR when we test it, as shown in Figure3-2.

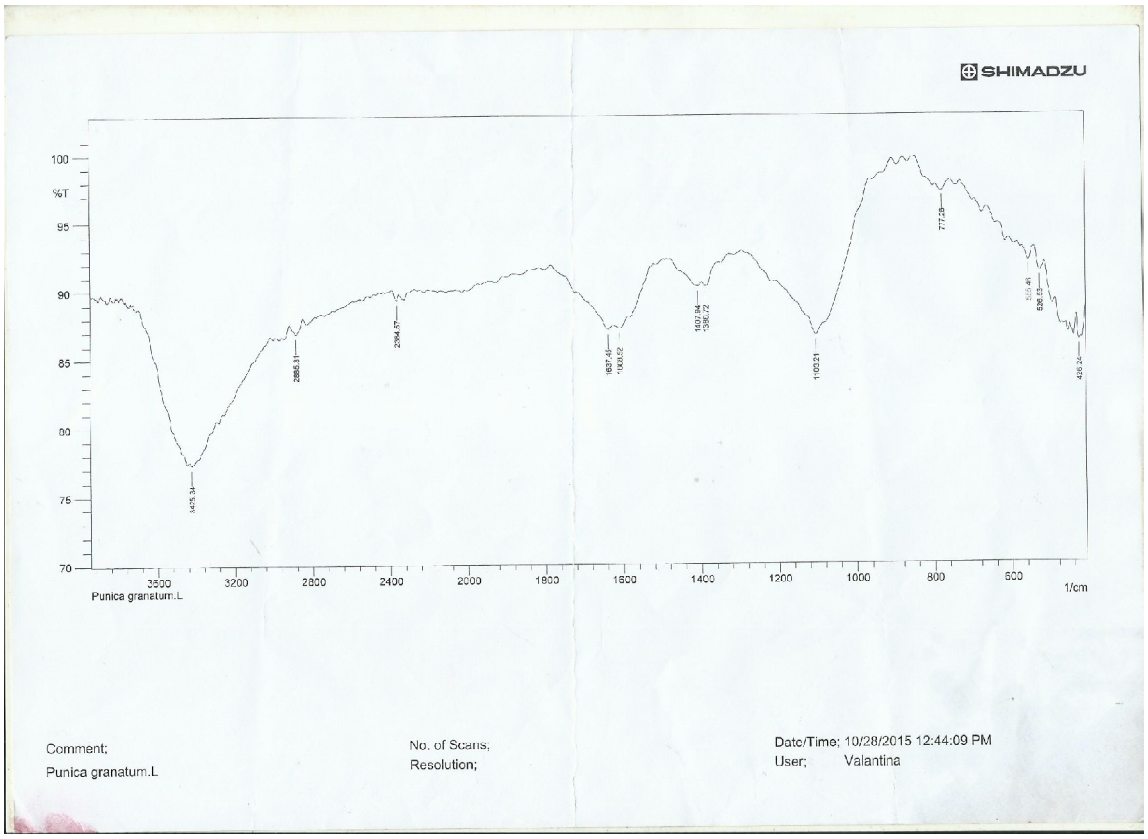


Fig3-2: IR spectrum

-At3500-3200 cm-1 refers to hydroxyl group. "Stretching vibration"

-At 1637.45-1608.52cm-1 refers to carbonyl group. "Stretching vibration"

-At 2885.31cm-1 refers to C-H (SP3) aliphatic "Stretching vibration".

-At 1407.94-1386.72 cm-1 refers to aliphatic C-H (SP3) "Bending".

-At 777.26 cm-1 refers to C-H aromatic.

3-3 UV/Vis Results

At maximum wave length =380 cm, the spectrum showed a hydroxyl groupin position 3 which reacted with the shift reagent sodium methoxide. And according to the results there is no hydroxyl group in position 7or position 4; so there is no reaction with boric acid**.**

Table 3-3: UV/Visabsorption

Sample	Abs
Sample+ NaOMe	0.279
Sample+ NaOAC	0.249
Sample+AlCl3	1.402
Sample+AlCl3+HCl	1.253
Sample+ boricacid	0.137

3-4 XRF Results

Table 3-4-1 XRF results

$ANALYSIS_RESULTS:

ROMAN [A0(RES)= 3203] WEIGHT [g/cm^2]:
.204

EL	E [KEV]	INT [C/S]	S	T	CONC [FRAC]	ERROR
K	3.312	0.392	3.27E+03	0.0406	1.42E-02	1.52E-02
CA	3.690	0.211	5.86E+03	0.0465	3.72E-03	4.01E-03
CR	5.411	0.035	9.24E+05	0.1270	1.42E-06	-LDL-
MN	5.895	0.057	4.51E+05	0.1598	3.79E-06	4.21E-06
FE	6.400	0.430	6.60E+04	0.1983	1.57E-04	1.69E-04
CU	8.041	0.882	1.09E+06	0.3392	1.14E-05	1.22E-05
ZN	8.631	0.303	3.35E+05	0.3905	1.11E-05	1.19E-05
PB	10.540	0.125	1.29E+06	0.5353	8.74E-07	9.42E-07
BR	11.907	0.185	1.73E+05	0.6144	8.40E-06	9.05E-06
RB	13.375	0.053	1.40E+05	0.6788	2.68E-06	-LDL-
SR	14.142	0.316	2.46E+05	0.7055	8.81E-06	9.46E-06

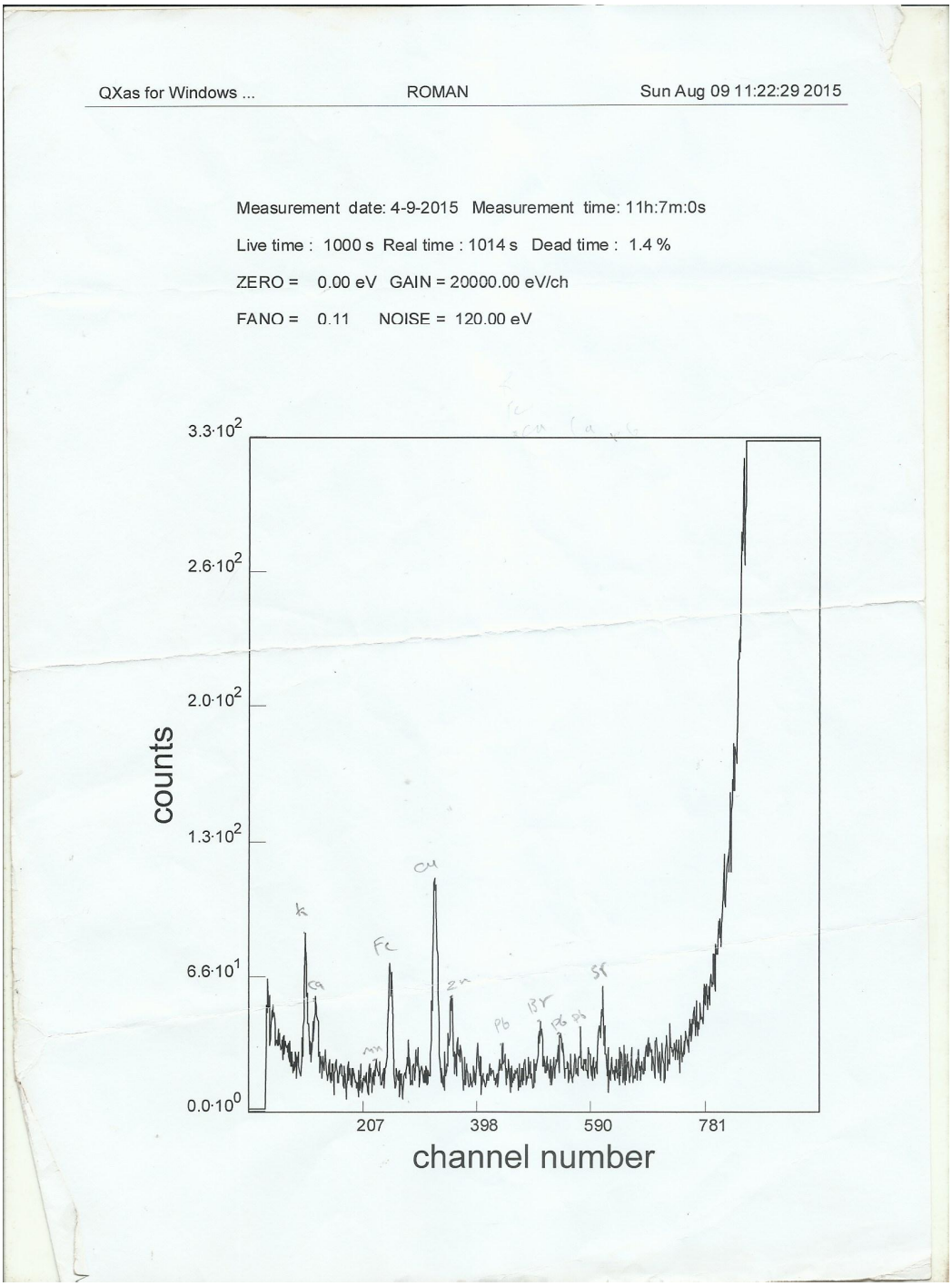


fig3-4 : XRF spectrum

XRF results showed that high concentration of potassium , calcium ,Iron . results showed low concentration of copper , zinc ,bromine , rubidium ,manganese , lead , chromium and stranchium.

Table3-4-2 : minerals concentration

Element	***Concentration(ppm)***	***Concentration%***
K	14200	1.42
Ca	3720	0.372
Cr	1.42	1.42E-4
Mn	3.79	3.79E-4
Fe	157	1.57E-2
Cu	11.4	1.14E-3
Zn	11.1	1.11-3
Pp	0.874	8.74E-5
Br	8.4	8.40E-4
Rb	2.6	2.68E-4
Sr	8.81	8.8E-4

XRF results showed that a high concentration of potassium , calcium ,Iron and a low concentration of copper , zinc ,bromine , rubidium ,manganese , lead , chromium and stranchium.

3-5 Determination of potassium using flame photometer

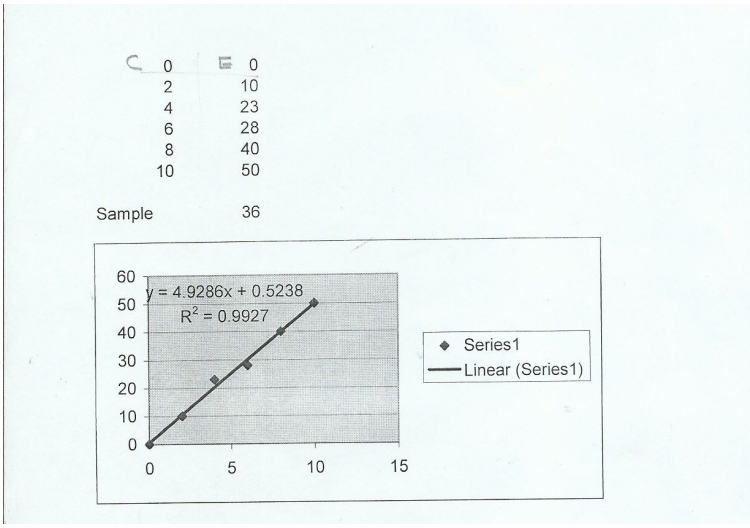


Fig3-5 : flame photometer results

The concentration of potassium was 89.96% in the hole content of pomegranate ash.

3.6 Conclusion

Flame photometer results showed that pomegranate fruit contain high concentration of potassium .

The results of analysis of pomegranate seeds showed that there is a high concentration of potassium , calcium and Iron in the seeds . Also showed a low concentration of lead , chromium , manganese , copper , zinc , rubudium , bromine and stranchium .

The results showed that pomegranate peels contain flavonoids and alkaloids .

According to the results of U.V/Vis and IR the type of flavonoid was flavonol.

Reference

[1]Agroforestg database 4.0 (orwaetal.2009).

[2] Agfact H3.1.2, first edition 1983.

j.f. Johnson / former principal Hortiulturist Division of plant Industries (Reviewed August 2002).

[3](Poyrazolglu and others 2002;Barzegar and other 2004).

[4]Aviram and other2002;Mirdehgane and Rahemi2007.

[5]Li and other 2006.

[6]Tanaka et .1986;wang et at 2004,2006.

[7]fruit,vegetables and cereal science and biotechnology2010,Global science book

[8]Erich Grotrewold ,department of celluar and molecular biology ,the science of flavonides,ohi state university Colambus,Ohiusa.

[9] Machado et al .2003 ,barga et al 2005.

[10] Zhang et al 1995,1997 .

[11]Toi et al 2003 .