Sudan University of Science and Technology

College of Science

Department of Chemistry

Study of the Major Flavonoid from *hydnora abyssinica* (Tartous)

دراسة للفلافونيد الرئيسي في نبات الترتوس

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قال تعالى :

((ربنا لا تؤاخذنا ان نسينا او اخطأنا ربنا ولا تحمل علينا إصرا كما حملته على الذين من قبلنا ربنا ولا تحملنا ما لا طاقة لنا به واعف عنا واغفر لنا وارحمنا انت مولانا فانصرنا على القوم الكافرين))

سورة البقرة الاية (286)

Dedication

To my great father and mother

Who gave me ahand to show the of live

To my lovely sister for

Their supporting and kindness

To my friend

who help me a lot.

To them all of dedicate this project as asign of thanks .

Appreciation respect and love .

Acknowledgment

First of all thanks our god for helping and supporting my research greatest thanks for our project supervisor (Mrs M-abd alkareem). Who has been agood supervisor and agood leader who show me the way for success .

My grateful and special thanks for My friends who help me in my research thanks my family teachers and friend .

Abstract

In this study phenolic compounds were extracted from ***hydnora*** (tartos) using 95% ethanol .

The crude extracts were subjected to thin layer chromatography using chloroform methanol water (4:3.5:1) for separation . In this way compound (I) was isolated from the crude of ***hydnora***

The IR spectrum gave the expected functional groups for compound (I) .

مستخلص الدراسة

استخلصت المركبات الفينولية في الترتوس بواسطة 95% ايثانول عن طريق كروماتوغرافيا الطبقة الرقيقة تم فصل المركب(I) من نبات الترتوس باستخدام الكلوروفورم والميثانول و الماء بنسب (4:3.5:1) كمذيب 0

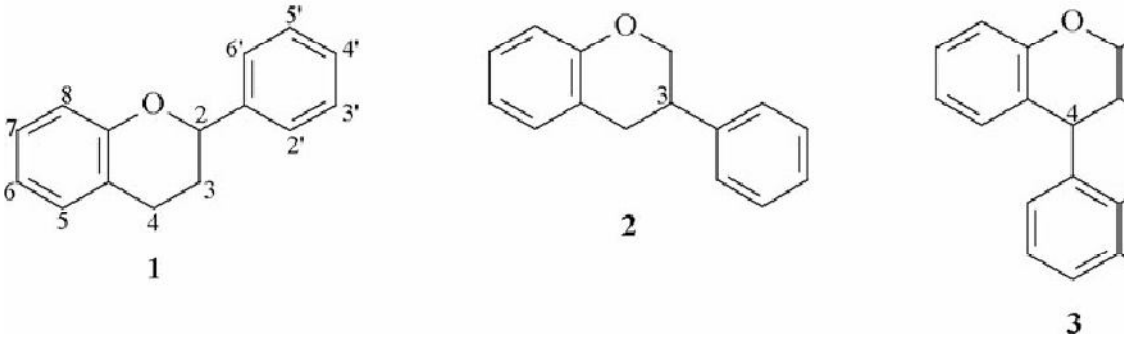
اوضح طيف الاشعة تحت الحمراء وجود الزمر الوظيفية المتوقعة0

1. INTRODUCTION

1.1General approach

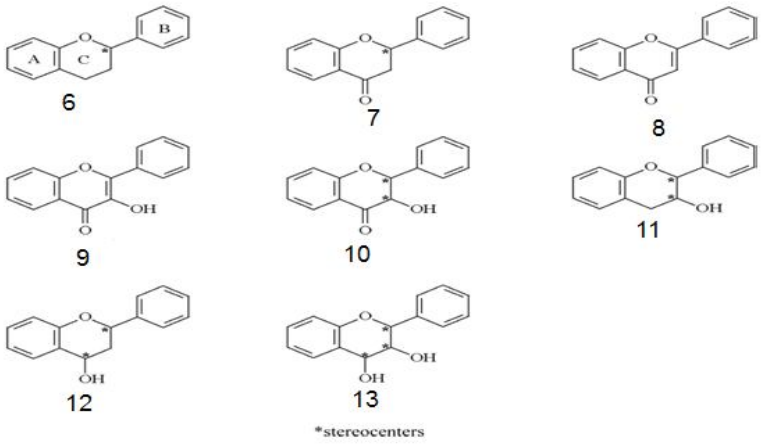
The study of flavonoid chemistry hasappeared, like that of most natural products, from the search for new compounds with useful physiological properties.Semisynthetic endeavors of oligoflavonoids are in most instances confined to those substitution patterns exhibited by monomeric natural products that are available in quantities sufficient for preparative purposes. In order to alleviate these restrictions,several programs focusing on synthesis of enantiomeric pure flavonoid monomers have been undertaken.However, synthesis of the desired enantiomer in opticallypure forms remains a daunting objective and is limited to only a few types of compounds. Chalcone epoxides α and β- hydroxydihydrochalcones, dihydroflavonols, flavan-3-ols, flavan-3,4-diols, isoflavans, isoflavanones, and pterocarpans
thus far have been synthesized in reasonable yields and purity.1

The term “flavonoid” is generally used to describe a broad collection of natural products that include a C6-C3-C6 carbon framework, or more specifically a phenylbenzopyran functionality. Depending on the position of the linkage of the aromatic ring to the benzopyrano (chromano) moiety, this group of natural products may be divided into three classes: the flavonoids (2-phenylbenzopyrans) **1**, isoflavonoids (3-benzopyrans) **2**, and the neoflavonoids (4-benzopyrans) **3**.Thesegroups usually share a common chalcone precursor, and therefore are biogenetically and structurally related 1



2-Phenylbenzopyrans (C6-C3-C6 Backbone)

Based on the degree of oxidation and saturation present in the heterocyclic C-ring, the flavonoids may be divided into : flavan(6),flavanone(7),flavone(8),flavanol(9),dihydroflavanol(10),flavan-3-ol(11),flavan-4-ol(12),flavan-3,4-diol (13).

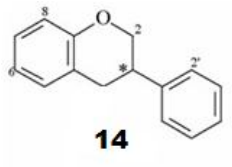


1.2 Iso flavanoids

The isoflavonoids are a distinctive subclass of the flavonoids. These compounds possess a 3-phenylchroman skeleton that is biogenetically derived by 1,2-aryl migration in a 2-phenylchroman precursor. Despite their limited distribution in the plant kingdom, isoflavonoids are remarkably diverse as far as structural variations

are concerned. This arises not only from the number and complexity of substituents on the basic 3-phenylchroman system, but also from the different oxidation levels and presence of additional heterocyclic rings. Isoflavonoids are subdivided into the following groups 1:

isoflavane(14),isoflavone(15),isoflavanone(16),isoflav-3-ene,isoflavanol(17),rotenoid(18),coumestane(19),3-aryicoumarin(20),coumaranochramene(21),coumaronochromone(22),petrocarpan(23)



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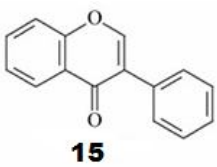

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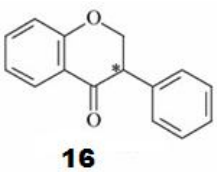

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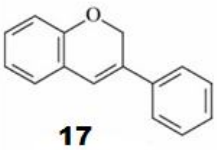

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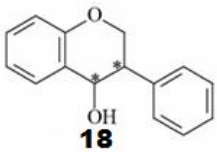

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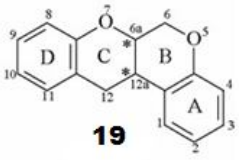

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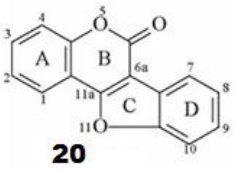

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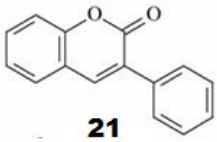

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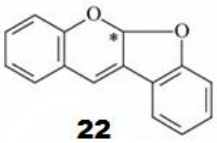

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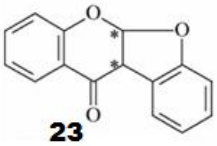

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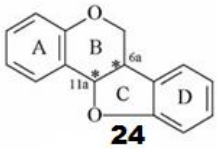

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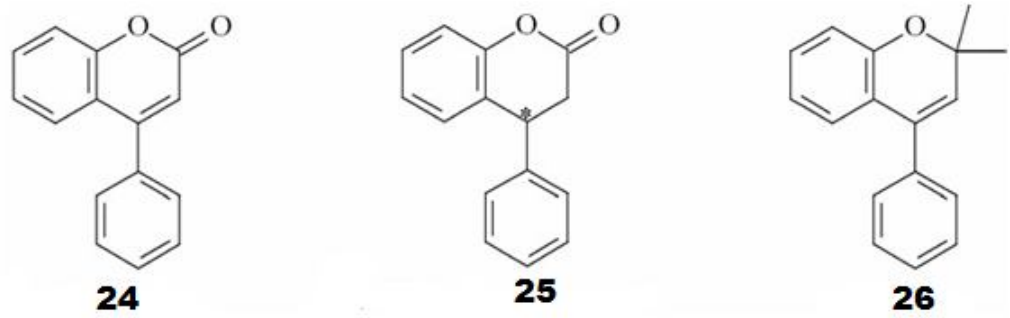
1.3 Neoflavonoids

The neoflavonoids are structurally and biogenetically closely related to the flavonoids and the isoflavonoids and comprise the 4-arylcoumarins(4-aryl-2*H*-1- benzopyran-2-ones)(24), 3,4-dihydro-4-arylcoumarins(25), and neoflavenes(26).



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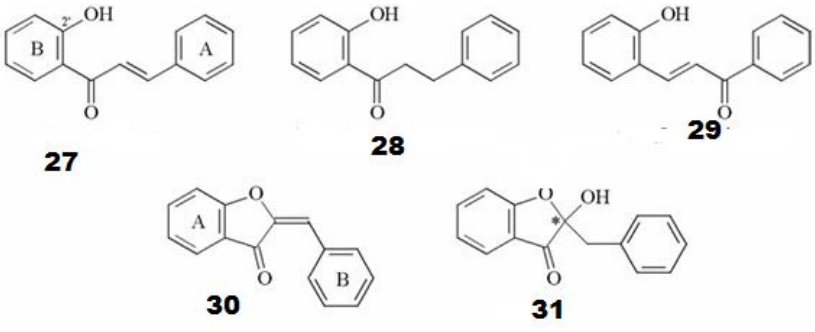
1.4Minor flavanoids

Natural products such as chalcones and aurones also contain a C6-C3-C6 backbone and are considered to be minor flavonoids. These groups of compounds include the2′-hydroxychalcones(27), ″-OH-dihydrochalcones(28), 2′-OH-*retro*- chalcone(29), aurones (2-benzylidenecoumaranone)(30), and auronols(31).



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1.5 Isolation and Identification of flvonoids

Flavonoids and their conjugates form avery large group of natural product found in many plant tissues. Where they are present inside the cells or on the surface of different plant organs. The chemical structure of this class of compounds are based onC6-C3-C6 skeleton .they differ in the saturation of the hetero ring C , in the placement of the aromatic ring B at position C2 or C3 of ring C . the flavonoids may be modified by hydroxylation,methoxylation or O-glycosylation of hydroxyl group as well as C-glycosylation directly to carbon atoms of the flavonoid skeleton in addition alkyl group(often prenyls) may be covalently attached to the flavonoid moieties. and sometimes additional rings are condensed to the basic skeleton of the flavonoid core. The last modification takes place most often in the case of isoflavonoids, where the B ring is condensed to the C-3 carbon atom of the skeleton.

Flavonoid glycosides are frequently acylated with aliphatic or aromatic acid molecules. These derivatives are thermally labile and their isolation and further purification without partial degradation is difficult.

The multiplicity of possible modifications of flavonoids result in more than 6,000 different compounds from this class were known in the end of the last century and this number continues to increase2.Condensed tannins create a special group of flavonoid compounds formed by polymeric compounds built of flavan-3-ol units.In the plant kingdom, different plant families have characteristic patterns of flavonoids and their conjugates. All these compounds play important biochemical and physiological roles in the various cell types or organs (seed, root,green part,fruit).where they accumulate. Different classes of flavonoids and theirconjugates have numerous functions during the interactions of plant with the environment, both in biotic and abiotic stress conditions 3Additionally, flavonoid conjugates, because of their commonpresence in plants, are important components of human and animal diet.Due to the different biological activities of plant secondary metabolites, their regular consumption may have serious consequences for health, both positive and negative4.For the mentioned reasons, methods for the efficient and reproducible analysis of flavonoids play a crucial role in research conducted in different fields of the biological and medical sciences.

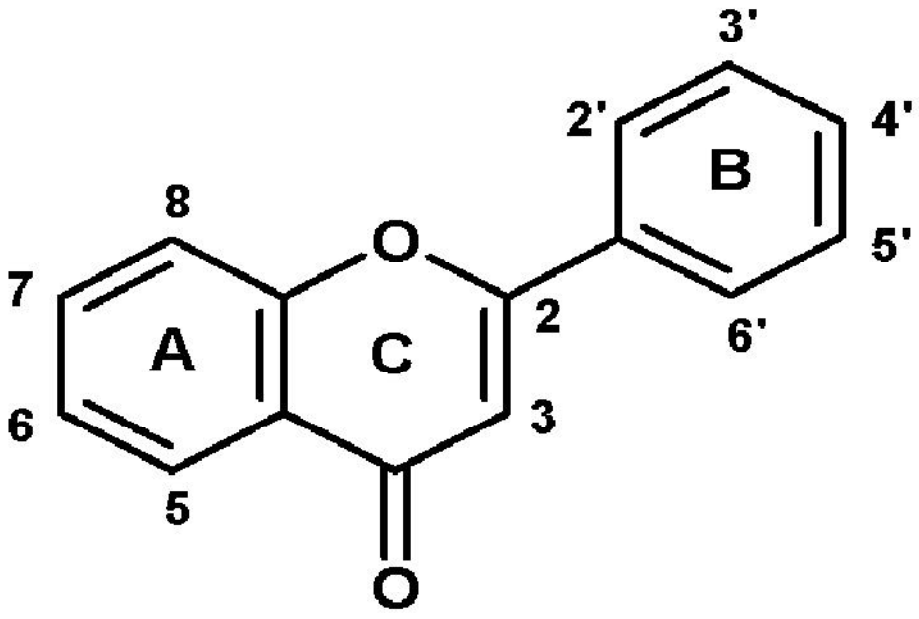


Fig. 1.Basic structure of flavonoid molecule.

The identification and structural characterization of flavonoids and their conjugates isolated from plant material, as single compounds or as part of mixtures of structurally similar natural products, create some problems due to the presence of isomeric forms of flavonoid aglycones and their patterns of glycosylation. A number of analytical methods are used for the characterization of flavonoids. In many cases, nuclear magnetic resonance (NMR) analyses (1H and 13C) are necessary for the unambiguous identification of unknown compounds; other instrumental methods (mass spectrometry, UV and IR spectrophotometry) applied for the identification of organic compounds fail to provide the information necessary to answer all the structural questions. Utilization of standards during analyses and comparison of retention times as well as spectral properties, especially when compounds are present in a mixture, is critical. An important area of research on flavonoids is the identification of their metabolites in animal tissues and body fluids (urine, blood, spinal fluid). For this, investigators have to deal with different modifications of the flavonoid moieties, modifications often not found in plant tissues 3.The metabolism of flavonoids in human and animal organisms, among others, is based on glucuronidation, sulfation, or methylation 4. The first two above-mentioned types of flavonoid aglycone modifications, occurring after their consumption by humans or animals, are lessoften found in the samples of plant origin.

1.5.1 Isolation of flavonoids and there conjugates from biological materials

The analysis of flavonoids and their conjugates is one of the most important areas in the field of instrumental analytical methods, helping to solve problems in biological and medical sciences.

Different methods of isolation of the natural products may be applied, and the utilization of various strategies is dependent on theorigin of the biological material from which the target natural products are to be extracted (plant or animal tissue or body fluids). In the case of polyphenolic compounds, it often is important to initially determine whether the researchers are interested in the identification of individual components present in a mixture of target compounds or whether they would like to estimate the total amount of phenolic compounds in the biological material investigated. This second approach most often takes place during the nutritional studies on different foods or fodders, mainly of plant origin.

The presence of carbohydrates and/or lipophylic substances may influence the profile of the qualitative and quantitative composition of flavonoids and their derivatives in the obtained extracts. One has to consider the above-mentioned. selection of the methods for sample preparation and extraction, and in many cases additional cleaning based on solid-phase extraction (SPE) of the extracted samples is required.

1.5.2 Preparation of Plant or Animal Tissue and Foodstuffs for Flavonoid Analysis

The uses of dried plant material for extraction may cause a substantial decrease in the yield of flavonoid conjugates. Acylated flavonoid glycosides are especially labile at elevated temperatures and are frequently

thermally degraded during the process of drying plant tissues. This is important during the profiling ofthis class of natural products in research directed toward the investigation of their physiological and biochemical roles in plants under the influence of environmental factors, or in studies of genetically modified plants for the elucidation of changes in metabolic pathways.

Free flavonoid aglycones exuded by plant tissues (leaf or root) may be washed from the surface with nonpolar solvents, such as methylene chloride, ethyl ether, or ethyl acetate. However, more polar glycosidic conjugates dissolve in polar solvents (methanol and ethanol), and these organic solvents are applied for extraction procedures in Soxhlet apparatus. Mixtures of alcohol and water in different ratios are applied for the extraction of flavonoids and their conjugates from solid biological material (plant or animal tissues and different food products).

The extraction efficiency may be enhanced by the application of ultrasonication 5 or pressurized liquid extraction (PLE), a procedure performed at elevated temperature ranging from 60oC to 200C 6.Supercritical fluid extraction with carbon dioxide also may be used procedures have to be carefully adjusted because of the possibility of thermal degradation of the flavonoid derivatives. In many cases, further purification and/or preconcentration of the target compound fraction is necessary. In these cases liquid–liquid extraction (LLE) or SPE are most commonly used. For estimation ofthe extraction yield it is necessary to spike biological materials with proper internal standards. Most suitable are compounds structurally similar to the studied analytes but not present in the sample. Compounds labeled with stable isotopes (2H or 13C) are useful when mass spectrometric detection is applied. In the case of theextraction of flavonoids from biological materials, different classes of phenolic compounds are often added. On the other hand, quantitative

analysis of consecutive components of the analyzed flavonoid mixture needs reference standard compoundsnecessary for preparation of calibration curves essential for a precise quantification.

The choice of the extraction procedure for obtaining flavonoid conjugates from biological material is very important and depends on the goals of the conducted research.The evaluation of the spatial distribution of target compounds on the organ, tissue, cellular, or even subcellular level is of special interest in some projects. In these situations, the amount of biological material for the isolation of natural products may be extremely small, and the application of microextraction techniques is necessary 7. In many cases, it is necessary to avoid the chemical and/or enzymatic degradation of the metabolites.This is of special importance in the profiling of flavonoid glycosides in research directed toward plant functional genomics or during physiological and biochemical studies that need information about all classes of flavonoid conjugates present, even the thermally labile acylated derivatives. On the other hand, in the phytochemicalanalysis of plant species or phytopharmaceutical studies of plant material, the repeatable isolation of all biologically active flavonoid aglycones with a good yield is more important. In these cases, more drastic extraction conditions are acceptable.

Robust multistep chromatographic methods are necessary for the isolation of individual components from plant extracts containing new uncharacterized compounds.Various stationary phases are used in column chromatography,including polyamide, Sephadex LH-20, and different types of silica gels.The proper choice of solvent systems is necessary, often requiring the application of gradients of more polar (normal phases) or more hydrophobic solvents (reverse phases), together with the above-mentioned chromatographic supports in different chromatographysystems.

minor flavonoid components are difficult to obtain as pure compounds. In cases of analysis of samples containing a number of compounds present in small amounts, the application of an analytical chromatographic systems enhanced by proper detectors (UV, NMR, and/or MS) gives spectrometric information sufficient forestablishing the structure of minor target components. When liquid chromatography is used for separation of compounds, multiple detector systems are available (UV diode array detector, mass spectrometers, and nuclear magnetic resonance spectrometer).

It is possible to achieve complete structural information aboutisomeric flavonoids and their conjugates in this way.

1.5.3 Preparation of Body Fluids

For the isolation of flavonoids and their derivatives from liquid samples likedifferent approaches are usually applied. The first one is based on liquid–liquid extraction and the second one on solid-phase extraction of target natural products mainly on RP C-18 silica gel cartridges. In the case of body fluids, special procedures have to be considered to avoid degradation of target compounds due tothe activity of different enzymes present 8. However, in some cases, flavonoid conjugates can be enzymatically hydrolyzed with external glucuronidases and sulfatases prior to the isolation and analysis of products.

1.6 Structural Characterization and /or Identification of Flavonoid and their conjugates

All physicochemical methods applied in the field of organic chemistry are useful for structural characterization or identification of individual flavonoids and their conjugates.

The separation approaches mentioned above may be consideredin different ways. The first one is directed toward the analysis of single compounds obtained after exhaustive isolation and purification procedures. The method of choice in this approach is NMR of 1H hydrogen and/or 13C carbon isotopes, dependent on the intensity of the interactions between different atoms within amolecule placed in a high-intensity magnetic field. Different NMR experiments have been developed to achieve information concerning chemical structure of the studied molecule on this basis.MS applied for the analysis of organic compounds utilize different ionization methods and may be equipped with different types of analyzers. In addition, these instruments may be combined with GC/LC or capillary electrophoresis (CE) apparatus.

However, simple chemistry based on single reactions such as silylation, methylation, and acetylation blocking polar functional groups has to be done on the studied samples prior to GC-MS analyses. Derivatization of polar groups improves structural information obtained from MS spectra and ameliorates the volatility of analytes, decreasing the thermal degradation of compounds within the GC capillary column.MS is a very sensitive analytical method used to identify flavonoid conjugates or to perform partial structural characterization using microgram amounts of sample9.significant structural data can be obtained from less than 1 mg of the analyzed compound when different MS techniques areused in combination with chemical derivatization of the characterized

compounds10.Flavonoid glycosides are thermally labile compounds and the evaporation without decomposition of the analyte is impossible.In this situation, soft ionization methods need to be applied for the analysis of this group of compounds, and the analyte molecules are ionized without evaporation in high vacuum (FAB or LSIMS, MALDI) or under atmospheric pressure (ESI, APCI). From normal mass spectra, information can be obtained about the molecular weight of the whole conjugate, the size of the sugar moieties attached to the aglycone, and the molecular weight of the aglycone11.

1.6.1 Identification of Flavonoid Conjugates in Mixtures

GC has been utilized for the separation of free flavonoids present in mixtures. This method offers a good efficiency of separation, but the necessity of derivatization of the compounds prior to the analysis decreases its applicability. Moreover, the derivatization procedures (e.g., methylation and trimethylsilylation) have their own limitations. When GC-MS is used, the mass spectra of silylated compounds are dominated by fragmentation products derivedfrom the elimination of silyl groups; thus, less structural information on the desired compound is obtained and the utilization of standards becomes necessary. On the other hand, some rearrangements may take place during the methylation of flavonoid –OH groups, and additional compounds become present in the reaction mixture after the derivatization. This is especially the case for flavanones, which rearrange to chalcones. However, mass spectra of methylated flavonols, flavones, or isoflavones give good structural information about the corresponding isomers. Thecomparison of mass spectra of silylated and methylated isoflavones from extracts obtained from lupine roots was previously demonstrated 12. It is possible to obtain additional information

when CD3I is used for the methylation of flavonoids.The chromatographic separation of the trimethylsilyl derivatives of components of a given mixture was better than that of the methylated compounds, but the mass spectra of methylated isoflavones provided more structural information.The two other chromatographic techniques, LC and CE, allowone to work without the chemical derivatization of analytes and permit the simultaneous separation of flavonoid aglycones and glycosidic conjugates. The combination of LC or CE with MS permits the use of different soft ionization methods.

Due to a wide range of biological activities of flavonoids consumed by humans and animals, there is a high interest in the metabolism of these compounds.

The most important groups of this class of natural products are the phytoestrogenes(isoflavones: genistein derivatives) and antioxidants (anthocyanins: flavonols and flavones); their interactions with proteins (tannins) and their metabolites aremonitored in physiological fluids (urine, blood, milk) and tissues 13. These kinds of studies will help to elucidate the influence of the flavonoids on human and animal health.

1.7Flavonoids in food

A ‘‘poor’’ diet is a major contributing factor to the etiology of chronic diseases such as heart disease and cancer. However, defining what constitutes a ‘‘healthy’’ diet remains contentious, as it is difficult to definitively ascribe beneficial and detrimental properties to the diverse

components of the many foods we consume. Nevertheless, considerable evidence indicates that adequate fruit and vegetable consumption has a role in maintaining health and preventing disease. Some of these protective effects may be due to flavonoids, which are widely distributed in plant-based foods at varying levels.(19)concentrations of flavonoids in foods can vary by many orders of magnitude due to the influence of numerous factors such as species, variety, climate, degree of ripeness, and postharvest storage.

1.7.1 Selection Of Foods And Flavonoids Classes

As all foods of plant origin potentially contain flavonoids(15, 16)and over 4000 individual compounds have previously been identified,(17) the development of a comprehensive flavonoid database is a huge task to undertake.in the first instance, it was decided to focus on foods commonly available and eaten in Britain and to initially consider those flavonoid classes that have attracted most attention in relation to potential health benefits namely flavonols, flavones, catechins, flavanones, and anthocyan(id)ins.(18)

1.7.2 Fruits

Catechins, flavonols, and proanthocyanidins are abundant in fruits. In contrast, flavanones and flavones are restricted to citrus varieties such as oranges and lemons In some fruits (e.g., apples), flavonols are principally present in the skin and hence peeling significantly reduces levels unlike catechins which are found in the flesh of fruits. Overall, catechins were the

most abundant flavonoid, ()-catechin (C) and (_)-epicatechin (EC) being particularly prevalent.Black grapes (4.9 and 4.7 mg/100 g, C and EC, respectively) are one of the richest fruit sources of catechins followed by apples (0.8 and 6.3 mg/100 g, C and EC, respectively). Catechins are also relatively abundant in stone fruits.Strawberries were found to contain the most complex mixture of catechins. Catechin esters have been characterized, but not measured, in some fruits such as nectarines and mangos.Type B procyanidins are also present in fruits, with apples (3.8 to 15.4 mg/100 g), plums (16.1 mg/100 g), and peaches (3.2 mg/100 g) containing the highest concentrations. However, citrus fruits do not appear to contain detectable levels of catechins or type B procyanidins.Quercetin is the most common flavonol in fruits Although kaempferol and myricetin have also been identified in fruits such as peaches and pears, concentrations are generally too low to be readily quantified in the whole fruit.

The skin of these fruits contains these flavonols in significant amounts; however, their flesh, which constitutes >70% of the fresh weight, does not. Consequently, when analyzed as normally eaten only trace levels are present.Often termed the citrus flavonoids, flavanones are only found in citrusfruits such as oranges, grapefruit, and lemons.

Although citrus fruit also contains low levels of flavones, the olive is by far the richest source of luteolin and apigenin (12.4 and 4.6 mg/100 g, respectively).**(19)**

1.7.3 Vegetables

varieties of vegetables and tomatoes (Lycopersicon species) are abundant sources of flavonols, primarily quercetin and kaempferol .Flavones are also found in some vegetables such as celery, sweet peppers, and lettuce.

Catechins and type B procyanidins, however, have not beenfound in leafy green or root vegetables but have been detected in legumes such as broad and green beans.

The tomato is the only vegetable (although taxonomically a fruit) to possibly contain the flavanones naringenin and hesperetin.Of the Allium species, shallots and red onions represent the richest potential source of quercetin containing 95 and 64 mg/100 g, respectively. Brassica vegetables including broccoli, kale, cabbage, and brussels sprouts tend to contain complex mixtures of flavonols, with significant quantities of kaempferol and myricetin glycosides present in addition to quercetin conjugates. Kale is a good example of this with mean levels of 11.5 mg quercetin/100 g and34.1 mg kaempferol/100 g. Legumes such as green and broad beans also contain complex mixtures, mainly of flavonols and catechins. For example, broad beans contain (_)-epicatechin (30.0 mg/100 g), ()-catechin (14.5 mg/100 g), (_)-gallocatechin (4.8 mg/100 g), and quercetin, myricetin, and kaempferol at concentrations below 3.0 mg/100g.Green chilli pepper is one of the few vegetables to contain both flavonols (quercetin, 11.39 mg/100 g) and flavones (luteolin, 2.7 mg/100 g) at detectable levels. Celery and sweet ball peppers are the main food sources of flavones independent of flavonols. (19)

1.7.4 Beverages

Catechins are often the most common flavonoids in beverages such as fruit juice, tea, and wine. These tend to contain complex mixtures of simple catechins and their gallated esters. Type B procyanidins have frequently been characterized in beverages such as fruit juices; however, reliable quantitative data are limited. Flavonols are also present in most beverages while flavanones are again restricted to citrus juices such as grapefruit and oranges.

The presence of flavones in beverages is not well described with only some characterization information available in the literature. Fruit juice contains both catechins and flavonols. Apple juice is one of the richest juice sources of catechins whereas cranberry juice contains the most flavonols, mainly in the form of quercetin and myricetin.

Tea is the only analyzed beverage to contain (_)-epigallocatechingallate (EGCG) in quantifiable amounts. EGCG and (_)-epicatechingallate (ECG) are the most abundant forms, each contributing 27% to the total catechin content (22.2 mg/100 ml) of black tea.

Three flavonols (quercetin, kaempferol, and myricetin) are also found in tea. For example, 100 g of decaffeinated tea contains 5.2 mg quercetin, 2.4 mg kaempferol, and 0.1 mg myricetin.Wine also contains a complex mix of catechins, flavonols, procyanidins, and flavanones.Red wine contains higher flavonoid levels than white or rose´ wines. Procyanidins usually represent 50% of the flavonoids found in red wine, followed by catechins (37%).

A similar profile is observed with beer where again procyanidins dominate accounting for 42% of total flavonoid content.(19)

1.7.5 Miscellaneous Food

Jam, confectionery, and herb compositional data are presented in Honey contains low levels of both flavonols and flavones, and the presence of the flavanone naringenin has also been documented. Fruit jams also contain low levels of flavonols and catechins, which generally reflect the flavonoid profile of the whole fruit.

Quantitative data for chocolate are limited, but the available literature demonstrates that it is a good potential source of ()-catechin, (_)-epicatechin, and type B procyanidins.Dark chocolate, for example,

contains 6.6 mg, 21.8 mg, and 2.1 mg/100 g of catechin epicatechin, and procyanidins, respectively.

Compositional analysis data for herbs are also limited; however, these plants may be rich sources of flavones. For example, parsley is the major source of apigenin (217.9 mg/100 g) in the whole database, while sage and thyme are rich in luteolin (33.4 and 39.5 mg/100 g, respectively).(19)

1.8 .Factors Affecting Flavonoid Content Of Food

the flavonoid content of fruits and vegetables analyzed in a particular study do not reflect ‘‘normal’’ levels in the products. Such regional differences are frequently cited in order to explain the apparent lack of association between dietary components and disease.(20 , 21)factors affecting flavonoid levels such as analytical variations, environmental factors, species characteristics, and the effects of processing and storage.

1.8.1 Analytical Variations

The methods of extraction and analysis can markedly affect the determination of flavonoids in foods.(22, 23, 24,25-29)Overall , sample preparation and extraction techniques along the linesdescribed by Merken and Beecher(24) were considered acceptable. These included freezedrying, extraction either with aqueous methanol containing an antioxidant such as solid-phase columns, filtration and the reduction of flavonol conjugates to the ‘‘free’’ aglycone by acid hydrolysis, or alkaline hydrolysis.Acceptable separation methodology normally involved RP-HPLC with UV, diode array, or electrochemical detection.

Fluorescence detection, capillary zone electrophoresis, and micellar electrokinetic capillary chromatography were also included if

identification and confirmation of eluted peaks was based on comparison with external standards, or if mass spectroscopy or nuclear magnetic resonance was used to confirm structural identity.

1.8.2Environmental Factors

The flavonoid content of plant foods may be affected by growing conditions.(15,16) For example, red wine produced in the warm, dry, and sunny conditions prevalent in the New World tend to contain more quercetin and myricetin (but less catechin) than the wines produced in the cooler and damper regions of Northern Europe.(30,31) Similar regional and climatic effects on flavonoid content have been observed for many different fruits and vegetables.(32,33-35) Concentrations of flavonol and flavanone monoglycosides, for example, are greatest on the surface of plants grown in or originating from arid and semiarid habitats.(17,33,36) However, flavonoid profiles are also influenced by irrigation, which, for example, modifies concentrations and types of anthocyanins and catechins in berries.(36,37)Marked differences in flavonoid content can even occur within a single variety depending on numerous factors such as maturity at harvesting, storage, use of glass and polythene, and organic cultivation methods.(38,32,39,40-42)Such environmental influences may account for why, for example, quercetin levels in Spanish cherry tomatoes range from 3.8 mg/100 g to 20.0 mg/100 g during a single year(34) and why produce grown in polythene tunnels with reduced UV exposure contain 98% less flavonoid glycosides than when grown in the open air.(16,17,43,36)

1.8.3 Species Characteristics

Computation of a single value from a wide range of sources will also minimize analogous confounding effects of varietal differences as flavonoid subclasses can vary widely between different cultivars of fruits and vegetables.(17,38,43,45) Examples of such differences include flavonols in berries,(46) flavones in honey(47) and olives,(48) catechins in pears(49) and apples,(39) procyanidins in apples(50) and blueberries,(51) and flavanones in citrus fruit(52) and grapefruit juice.(53) Typically, for example, the flavonol content of 12 chilli-pepper varieties ranges from 0 to 85 mg/100 g.(54) Such differences can be ascribed to: (a) genetic mutations influencing the synthesis and accumulation of flavonoids in tissue(17,33); (b) the degree of pigmentation, (73,33,46,55-57)particularly in berries(58) and onions(15) (although this has been recently disputedas original determinations may have included the nonedible and anthocyanin-rich skin of the onion); (c) the stage of maturity.

the nature of the sugar attachment may influence the bioavailability of a particular flavonoid(59,60)it may ultimately be preferable for the to show flavonoid content as glycosides.

1.8.4 Processing and Storage

industrially produced products such as tea, red wine, and fruit juice have significantly different flavonoid levels and profiles than the original fresh product.(61 – 65) Processing and preservation can expose fresh products to increased risk of oxidative damage and the activation of oxidative enzymes such as polyphenol oxidase.(49,66) procedures such as solvent extraction, sulfur dioxide treatment, pasteurization, enzymic clarification, heating, canning, irradiation, drying, and fermentation have been reported to affect procyanidin and catechin concentrations in fruit juice,(61-63,67-70) quercetin glucosides, catechins, and procyanidins in grapes,(71) procyanidin and flavonol levels in tomatoes and related sauces,(72) and quercetin concentrations in berries.(35)

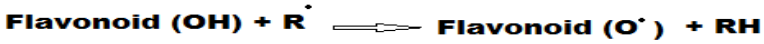
1.9 biological activities of flavonoids

Anti-oxidant and anti-free radical activities of flavonoidsThe most described property of flavonoids is their capacity to protect the organism against free radicals and oxygenated reactive species (ORS) produced during the metabolism of oxygen(73). The cellular damage by the free radicals causes a change of the net charge of cells, thus modifying their osmotic pressure and inducing their swelling and their death.

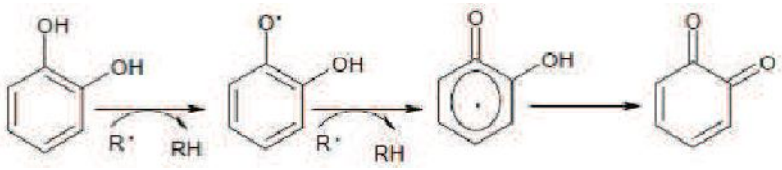
The free radicals act also on the mediators of the inflammatory diseases, and accelerate the tissue damage. Moreover, cells lesions lead to an increace in the production of the ORS which induces the consumption and the depletion of the endogenous chelating agents. To protect against species, the organism and living cells have developed several mechanisms (74)including enzymes like the superoxyde dismutase, the catalase and the

glutathion peroxidase, and also non-enzymatic homologues such as the glutathion, the ascorbic acid and l'α-tocopherol.

The protective effect of flavonoids is due to several mechanisms such as free radicals trapping, enzymes inhibition and metallic ions chelation. These properties depend on the structure of the flavonoids and the degree of substitution and saturation The flavonoids can prevent the damage caused by the free radicals according to various ways; one of them is the direct trapping of the radicals. In this case, the flavonoids are oxidized by the radicals (R•) leading to less reactive and more stable species according to the following mechanisms (74)



The formed flavonoxy radical is stabilized by resonance. The non-paired electron can be delocalized on the whole of the aromatic cycle. But, it can continue to evolve according to several processes (dimerisation, dismutation, recombination with other radicals, oxidation in quinon) either while reacting with radicals or other antioxidants, or with biomolecules. The flavonoxy (FL-O•) radical can react with another radical to form stable quinine as follows:



The flavonoxy radical can interact with oxygen to give a quinone and a superoxide anion.This reaction is responsible for an undesirable prooxidant effect of flavonoids. So the capacity of flavonoids to act as antioxidant depends not only on the redox potential of the couple Flavonoid (O.)/ Flavonoid (OH), but also on the reactivity of generated flavonoxy radical(75)

1.9.1 Effect on the mediator of nitric oxide synthesis

Several flavonoids reduce the cellular lesions related to ischaemia, by interfering with the activity of nitric oxide synthase. as the endothelium cells and the macrophages. The nitric oxide is produced through theThe nitric oxide is produced by various types of cells such constitutive activity of nitric oxide synthase. It plays a role for the maintenance of thedilation of the blood-vessels, the relaxation of the smooth muscles, the signal of transduction and the inflammation(76 – 77). However, at high concentrations it induces an irreversible oxidative damage on cellular walls; because the activated macrophages increase their simultaneous productions of nitric oxide and the superoxide anions. The nitric oxide reacts with the free radicals producing peroxynitrite anion (ONOO-), a more reactive species.

When the flavonoids are used as antioxidants, the free radicals are trapped thus reducing the conversion of nitric oxide into peroxynitrite(78). Flavonoids can also react with nitric oxide directly (75). Therefore, it was speculated that the trapping of nitric oxide by the flavonoids is in the origin of their protective effect of the cardiovascular system.

1.9.2 Inhibition of the enzymes activities

It is well known that flavonoids are able to inhibit the activities of several enzymes implicated in radical's generation. Among these enzymes, the xanthine oxidase, lipoxygenase, cyclo-oxygenase, peroxidase and tyrosin kinase are the most studied.

The xanthine dehydrogenase and the xanthine oxydase are implied in the metabolism of the xanthine into uric acid. The xanthine deshydrogenase is the configuration available under the normal physiological conditions, but it changes into xanthine oxydase during cells reperfusion (reoxygenation) and reacts with molecular oxygen to release the superoxyde radical (O2-).

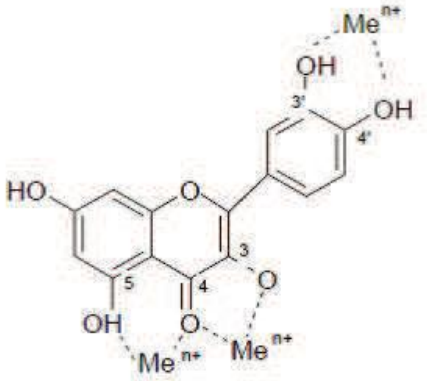
The flavonoids act as a strong inhibitor of the xanthine oxydase and as a trapper of the superoxide radical(79).The flavonoids have also the capacity in one hand, to inhibit the metabolism of the acid arachidonic (80)by inhibiting the lipooxygenase and thus preventing the production of the chimiotactic compounds from this acid.

This characteristic gives to the flavonoids the anti-inflammatory and anti-thrombogenic properties. In the other hand, flavonoids have also the capacity to reduce the release of peroxidases and proteolytic enzymes and thus the production of the ROS(81) .

The activity of the tyrosin kinase is affected by the presence of the flavonoids(82) . This enzyme is implied in several cellular functions such as the enzymatic catalysis, the transport through the membrane, the transduction of the signals for hormones or growth factors and the transfer of energy in the synthesis of ATP. So, the inhibition of this enzyme by the flavonoids interferes with the way of transduction of the signals controlling the cellular proliferation.

1.9.3 Chelation of the metal ions

The ions of iron (Fe2+) and copper (Cu+), are essential for certain physiological functions of living cells(75). They can be, either as components of hemoproteins, or of cofactors of various enzymes implicated in antioxidant defense system of cells. Besides their beneficial role, they are also responsible for the production of the hydroxyl radical by the reduction of hydrogen peroxide (.OH) The flavonoids form a stable complex with transition metals (Fe3+, Al3+, Cu2+, Zn2+); the stoichiometry of the complex and the site of chelation depend on the nature of the flavonoid mainly the presence of the catechol part and the pH (83- 84) .Moreover, this phenomenon of chelation is accompanied sometimes by the oxidation of the flavonoid (Cu2+, Fe3+). The chelation is occurred generally on the hydroxyl groups in position 3' and 4' of the B cycle, on the position 3 of hydroxyl group of A cycle and on the positions 3 and 4 of carbonyl group of C cycle



When the flavonoids have several chelating metal sites, they can be polymerized. The copolymerization of the flavonoids and iron is responsible for anemia disease observed in large consumers of tea (85) .

The capacity of the flavonoids to complex metals is probably at the origin of the inhibition of many enzymes whose active site containsmetals.

1.9.4 Cardiotonic activity:

Flavonoids have been reported to have action on the heart. The unsubstituted parent flavone exerts coronary dilatory activity and was commercially available under the name 'Chromocor' and its combination with routine and isoquercetin was also available with brand name 'flavoce', useful in the treatment of atherosclerosis.

3-methyl quercetin has positive chronotropic effect on guinea pig right atrium and antiarrhythmic effect on left atrium. In recent report the cardiotoxicity (negative inotropic effect) of doxorubicin on the mouse left atrium has been inhibited by flavonoids, 7-monohydroxy ethyl rutoside and 71,3',4'- trihydroxyethyl rutoside. In a recent review Huesken *et al*. gave detailed discussion on the cardioprotective effectsof flavonoids**86**.

The glycosides of luteolin, apigenin and genistein produced antihypertensive activity even more than the reference drug papaverine. Three flavonoids showed vasorelaxant effect in order of potency, luteolin > eriodictyol > naringenin on rat thoracic aorta.

Luteolin, apigenin and genistein exhibited their action through different mechanisms by inhibition of the calcium release from sarcoplasmic reticulum, enzymatic systems such as protein kinase-C and thecalcium influx.

Different flavonoids were tested for a positive inotropic effect on guinea pig papillary muscle placed at 0.2 Hz in a Kerbs-Henseleit solution at 30° C.Quercetin showed the most potent intrinsic activity, and produced the strongest inotropic responses among the different flavonoids.The relative order of potency of the tested flavonoids was,

quercetin> morin = kaempferol > luteolin = apigenin> fisetin = galangin**87**.

1.9.5 Lipid lowering activity:

Oxidative modification of low-density lipoproteins(LDL) by free radicals is an early event in the pathogenesis of atherosclerosis.The rapid uptake of oxidatively modified LDL *via* a scavenger receptor leads to the formation of foam cells. Oxidized LDL also has a number of other atherogenic properties.

A number of mechanisms are likely to contribute to inhibition of LDL oxidation by flavonoids. Flavonoids may directly scavenge some radical species by acting as chainbreaking antioxidants**88**.In addition, they may recycle other chain-breaking antioxidants such as α-tocopherol by donating a hydrogen atom to the tocopheryl radical.Transition metals such as iron and copper are important pro-oxidants, and some flavonoids can chelate divalent metal ions, hence preventing free radical formation. The ability of quercetin, and the quercetin glycosides, to protect LDL against oxidative modification has shown a significant protective effect. Liquiritigenin showed a significant fall in serum cholesterol, LDL-cholesterol and atherogenic index. Influence of flavonoids on blood coagulation has been studied. The anticoagulantaction of heparin was antagonized by flavonoids extracted from *T. hircanicum*. Ability of different flanovoids to inhibit the procoagulant activity of adherent human monocyties has been studied recently and hinokiflavone, a bioflavonoid has been found to be very most active in inhibiting the interleukin induced expression of tissue factor on humanmonocytes. Silymarin counteracts ethanol-induced lipid peroxidation injury by reducing liver MDA and raising GSH levels**89**.

1.9.6 GIT:

a) Antiulcer Activity:

Some recent reports have indicated that many flavonoids possess antiulcerogenic activity. Oral treatment with the ether fraction of the flavonoid extract demonstrated a good level of gastric protection.Mucous content was increased and accompanied by proportionate increase in proteins and hexosamines.β-Hydroxy ethyl rutosides, gossypin,naringin, naringenin and (+)-Cyanidanol-3 were shown to exhibit anti-ulcer activity 89.

Quercetin, rutin and kaempferol administered intraperitoneally (25-100 mg/kg) inhibited dose-dependent gastric damage produced by acidified ethanol in rats. Flavone was inactive while naringin was active at a higher dose (200 mg/kg). Quercetin, kaempferol, morin, myricetin and rutin when tested were found to inhibit the mucosal content of platelet activating factor(P AF) in a dose dependent manner suggesting that the protective role of these substances may be mediated by endogenous PAF. Qurcetin, kaempferol, rutin produced an inhibitory effect on intestinal functions, and that their actions are mediated throughα_2-adrenergic and calcium systems**90** . This result mayα_2-adrenergic and calcium systems. This result may show the beneficial effects in diarrhea and other intestinal secretions. (+)-Cyanidanol-3 has histidine decarboxylase inhibitory activity and hence anti-ulcer activity**91** . 3-Methoxy-5,7,3',4'-tetra hydroxy flavan (Meciadanol), a congener of (+)-cyanidanol-3 exhibited significant anti-ulcer activity in pylorus ligated rats, restraint ulcers and gastric mucosal damage induced by aspirin models**89**.

b) Hepatoprotective activity:

Many flavonoids have also been found to possess hepato-protective activity. In a study carried out to investigate silymarin, apigenin, quercetin and naringenin as putative therapeutic agents against microcrystin LR-induced hepatotoxicity, silymarin was found to be the most effective one. Rutin and venorutin showed regenerative and hepato-protectiveeffects in experimental cirrhosis**91**. The results of several clinical investigations showed the efficacy and safety of flavonoids in the treatment of hepato-biliary dysfunction and digestive complaints, such as sensation of fullness, loss of appetite, nausea and abdominal pain. Silymarin normalizes cell phospholipid synthesis without showing any demonstrable effect on undamaged cells where by counteracting fattyliver. Moreover, earlier findings on a hepato-protective effect and the prevention of NSAIDs-inducedgastropathy may be confirmed**92,93**.

1.9.7 Effect on heat shock proteins:

Heat shock proteins (HSP) have been recognized against physiological stress such as heat shock, heavy metals and glucose starvation. Recent progress has revealed the role of HSPs in various diseases. HSP27 has been shown to be involved in the acquired resistance of tumour cells, hyperthermic and chemotherapeutic treatment. Aberrant expressionof HSP could cause various autoimmune diseases. Flavonoids inhibited the expression of HSP27, HSP47, and HSP72/73 **94**.

The results suggested the pharmacological possibilities of flavonoidsin diseases derived from abnormal expression of JISPs.

1.9.8 Anti-inflammatory activity:

A number of flavonoids are reported to possess antiinflammatory activity. Hesperidin, a citrus flavonoid possesses significant antiinflammatory and analgesic effects.Recently, apigenin, luteolin and quercetin have been reported to exhibit antiinflammatory activity. Quercetin, gallic acid ethyl ester and some as yet unidentified flavonoids might account for the antinociceptive action reported for the hydroalcoholic extract of *Phyllanthus caroliniensis*.Treatment with silymarin demonstrated reversal of the carrageenin induced biochemical changes. Detailed biochemical to studies establish mechanism of action of flavonoids have been carried out **95,96**.

1.9.9 Antineoplastic activity:

Quite a number of flavonoids have exhibited antineoplastic activity. Recent reviews have highlighted this activity**97**.Detailed studies have revealed that quercetin exerted a a dose dependent inhibition of cell growth and colony formation.The flavonoids kaempferol, catechin, toxifolin and fisetin also suppressecell growth. On screening antileukaemic efficacy of 28 naturally occurring and synthetic flavonoids on human promyelocytic leukaemic HL-60 cells, genistein, an isoflavone was found to have strong effect.

Genistein is also reported to inhibit in a dose dependent manner the growth of HGC-27 cells derived from human gastric cancer. Of the 14 flavonoids tested against murine and human cancer cell lines, 2',6'-diacetoxy -4,4' -dimethoxydihydro chalcone was the most potent and showed selectivity for the cell line P-388. Trifolirhizin tetraacetate showed greater selectivity for the human cell lines.

1.9.10 Effects on blood vessels:

Quercetin and rutin have been used as effective constituents of several pharmaceuticals used for treatment of capillary fragility and phlebosclerosis. The activities of certain flavonoids in inhibiting capillary permeability and Arthus phenomenon were found to be in the following order, hesperitin > rutin > quercetin> naringenin > kaempferol > isoquercitol**98** . It has been suggested that flavonoids, which contain free hydroxyl groups at 3, 3' and 4' positions exert beneficial physiological effects on capillaries.

Flavonoids tangeratin, hesperidin, quercetin, and rutin have been found to reduce aggregation of horse erythrocytes.

The decrease in blood cell aggregatio produced by most of the flavonoids may explain the reported beneficial effects of these compounds on abnormal capillary permeability and fragility, the reduction of disease symptoms and their protection against various traumas and stresses. The flavonoids O-(β-hydroxyethyl) rutoside, (+)- catechol, trihydroxyethylrutoside increased the negative charge density of the blood vessel wall *in vitro* and were markedly antithrombogenic. Quercetin also has been reported to inhibit aggregation of human platelets by several authors. Other antiaggregatory flavonoids reported were 3-methyl quercetin, toxerutin, fisetin, dihydroquercetin and flavone. Nobeletin and sinensetin decreased erythrocyte aggregation and sedimentation *in vitro* and might be useful in dietary control of high blood viscosity syndrome **99** .

Orally administered flavonoids weakly inhibit the vascular permeability and prevent pulmonary haemorrhage Acacetin at 25-100 mg/kg oral dose to mice reduced capillary fragility and at 50-100 mg/kg it reducedvascular

permeability **99**. Patuletin reduced the capillary permeability and was also reported to have antispasmodic and hypotensive effects.

1.9.11 Antimicrobial activity:

Flavonoids and esters of phenolic acids were investigated for their antibacterial, antifungal and antiviral activities. All samples were active against the fungal and gram-positive bacterial test strains and most showed antiviral activity**100**.

i) Antibacterial Activity:Antibacterial activity hasbeen displayedby a Twenty-five out of one hundred and eighty two flavonoid studies were found to be active against many bacteria.
Most of the flavonones having no sugar moiety showed antimicrobial activities where as none of the flavonols and flavonolignans tested showed inhibitory activity on the microorganisms**100**.

ii) Antifungal Activity: Number of flavonoids isolated from peel of tangerine orange, when tested for fungistatic activity towards Deuterophoma tracheiphila showed promising activity. Chlorflavonin was the first chlorine-containing flavonoid type antifungal antibiotic produced by strains of *Aspergillus candidus***101**.

iii) Antiviral Activity: Flavonoids also displayed antiviral, including anti-HIV activity. It has been found that flavonols are more active than flavones against herpes simplex virus type 1 and the order of importance was galangin > kaempferol> quercetin. a natural plant flavonoid polymer of molecular weight 2100 Daltons was found to have antiviral activity

against two strains of type-1 herpes type simplex virus, including a thymidine-kinase deficient strain and type -2 herpes simplex virus. Out of twenty-eight fiavonoids tested, flavan-3-o1 was more effective than flavones and flavonones in selective inhibition of HIV-1, HIV-2 and similar immunodeficiency virus infections**102.**

1.10 Effects of flavonoids consumption on biological activities.

Consumption	**Effect**
Quercetin –rich fruits and vegetables (during 15 years)	Decrease of apoplexy incidence
Green tea (during 10-11 years)	Decrease of cancers risk (lung, liver, colon) Retard cancers progression Decrease of breast cancer risk
Dadzein, genistein and coumestrol -rich food	Decrease of prostate cancer risk
375mg of curcumin (3 times a day for 12 weeks)	Effective treatment of anterior uveitis Increase of blood levels of glutathione peroxidase
55mg/day of isoflavonoids for 8 weeks	No effect on lipid peroxidation
Extract of red vine leaf (360 or 720 mg/day for 12 weeks)	Small effect on chronic venous insufficiency
Green tea extract + linoleic acid (3g/day for 4weeks)	Decrease of blood levels of malondialdehyde No effect on other markers of oxidative stress or production of nitric oxide
Red vine phenolic compounds (3time 660mg/day for 2 weeks	Increase in serum antioxidant capacity

Blackcurrant and apple juices (750 up 1500ml/day for one week)	Decrease of blood levels of malondialdehyde Increase in glutathione peroxidase (no other changes in antioxidant status)
Green or red tea (2g/day for 2 days)	Transient increase in blood antioxidant parameters (radical scavenging)
Flavonones, vitamin C	Decrease of prostate cancer risk
Lyophilized grape powder (flavans, anthocyanins, qercetin, myricetin, kaempferol, resveratrol)	Reduction in plasma triglyceride concentration, cholesterol (LDL), apolipoproteins B, E and TNF α
Quercetin	Reduced risk of mortality due to ischemic heart disease Reduced risk of lung cancer
Quercetin, naringenin, hesperitin	Reduced risk of breast cancer
Quercetin, myricetin	Reduced risk of asthma
Kaempferol, naringin, hesperitin	Reduced risk of type 2 diabetes
Myricetin	Reduced risk of cerebrovascular diseases

2- Experimental procedure :

The plant was identified by my project supervisor (Mrs M-abd alkareem).

2-1 Sample Collection:

The plant *hydnora abyssinica (*Tartous)was collected from Aljazzerastate.

2-2 Preparation of the Sample:

The roots of hydnora were air-dried after collection . The dried roots were ground into fine powder and then weighed

.

2-3 Extraction of the Sample:

Solvent-solid extraction was carried out on the weighed, air-dried and roots of hydnoraThe weighed sample was soaked with ethanol (95%) for two days , with continous shaking . The separation of the residue from filtrate was done by using filter paper. It was followed by the concentration of the filtrate by using distillation method .

2-4 Chromatography:

The type of chromatographic method was used to separate the constituents that were present in the hydnora extract is thin-layer chromatography.

2-4-1 Thin-Layer Chromatography (TLC):

TLC was used to ascertain the number of constituents present in the extract and to determine their purity. TLC was also used to determine the solvent mixture that will affect the separation of the components. The TLC were eluted with chloroform :Methanol : water (4 : 3.5 : 1) .

-Preparation of Silica Gel Plates:

50g of silica gel powder was weighed into a conical flask, 100ml of distilled water was added and the resulting solution was shaken vigorously in order to avoid lumps. The white smooth paste mixture was spread over the glass plate and was allowed to solidify. The coated glass plates were put inside oven for 1-2 hours at 1100C to ensure further solidification.

-Spotting of the Plates:

This is done with aid of capillary tubes to introduce few drops of the dissolved sample extract into the coated plate, allowing each drop to dry before adding another drop.

-Developing of the Plates:

After the solvent had travelled some distance across the plate, the plate was removed and allowed to dry and then spray a clouration reagent which is vaanalin solution with concentrated sulfuric acid . The separated components appeared as dark braown-red spots .

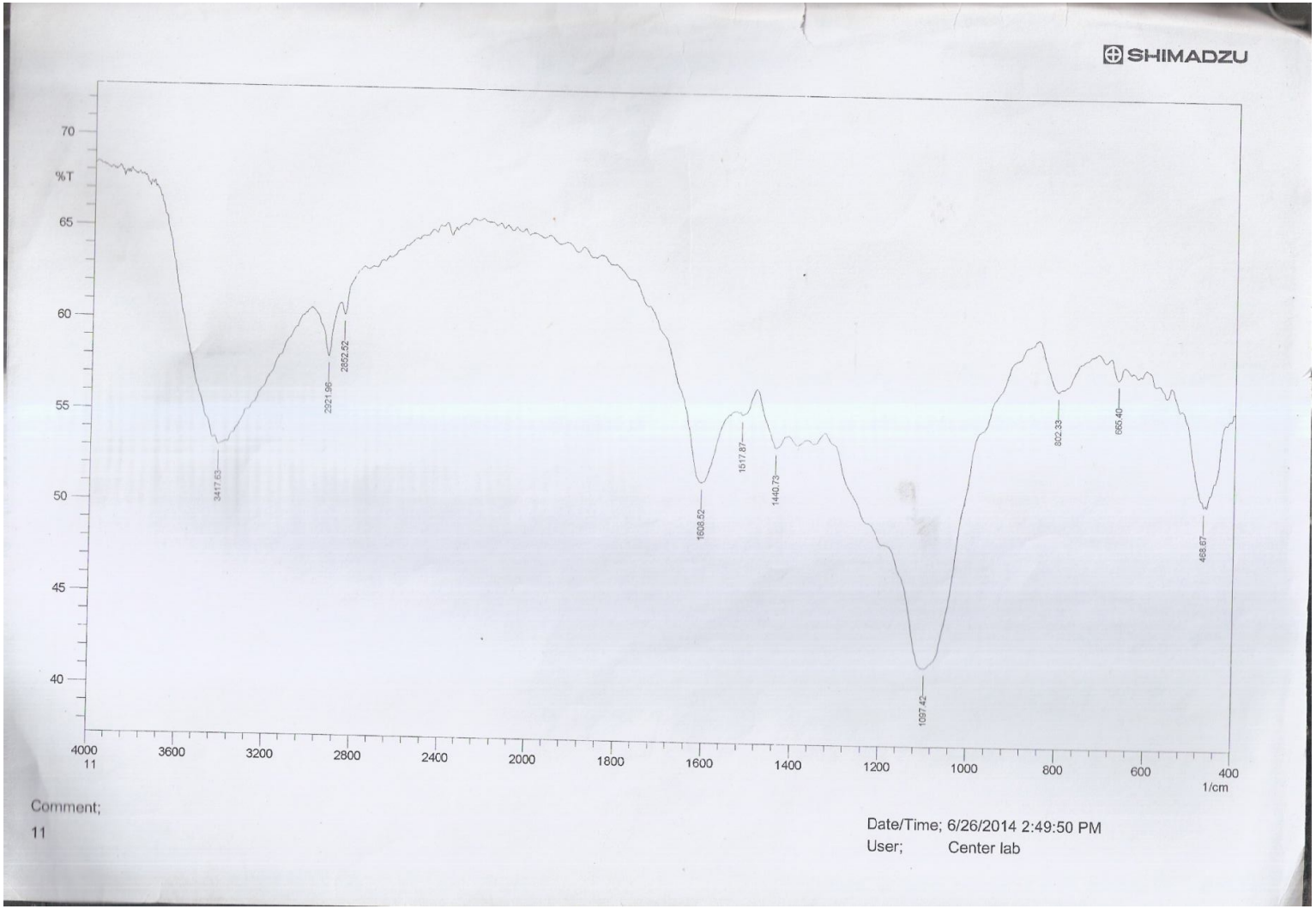
3- Results and Discussion

The Flvonoid of *hydnora* was extracted by ethanol after removing the solvent .acrude product was obtained which was fractionated by TLC (silica gel / 60 % acetic acid) .

After the usual work up apure flavonoid – compound was isolated .

In the IR spectrum (Fig I) it gave ~ (KBr) 665.40 -802.33 (C-H , Ar) , 1097.42 (C – O) , 1440.73 – 1608.52 (C = C , Ar) , 3417.63 (OH) .

Since the IR revealed absence of (C = O) Str . vib then this flavonoid is either anthocyanin or flavan .


SHIMADZU
70
%T
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45
40
3417.63
2921.96
2852.52
1608.52
1517.87
1440.73
1097.42
802.33
665.40
468.87
4000
3600
3200
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Figure 1: Explain the infra red spectrum of the compound (flavan or anthocyanin).

4. Refrence

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