Chapter 2

Isolation and Separation of Phenolics using HPLC Tool: A Consolidate Survey from the Plant System

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Abstract

HPLC is a versatile tool for separation of phenolics from the plant systems. Many studies are conducted for separation of phenolics using HPLC tool. This chapter summarized the work done in this area using various solvents, plant parts and assay condition in tabulated form.

1. General Introduction

In recent times, one of the key interests in food science and technology is the extraction, identification, and characterization of novel functional ingredients of natural origin. These ingredients are used as natural preservatives against food degradation, health promotion activities and value addition. Plants produce an amazing diversity of low molecular weight compounds. Although the structures of close to 50,000 have already been elucidated [1]. There are probably hundreds of thousands of such compounds. Only a few of these are part of 'primary' metabolic pathways (those common to all organisms). The rest are termed 'secondary' metabolites [2].

Amongst this diverse pool of metabolites, polyphenols are aromatic hydroxylated compounds, commonly found in vegetables, fruits and many food sources that form a significant portion of our diet, and which are among the most potent and therapeutically useful bioactive substances. The plant phenolics play important role in many physiological functions like, protein synthesis, nutrient uptake and oxidative enzyme (peroxidases) activities [3]. Photosynthesis and structural components. In addition, they also provide defense against microbial attacks and by making food unpalatable to herbivorous predators [4]. Thus, phenolics are overall important in many growth and development activities of the plants.

Besides the importance for the plant itself, such metabolites determine the nutritional quality of food, colour, taste, smell, antioxidative, anticarcinogenic, antihypertension, anti-inflammatory, antimicrobial, immunostimulating, and cholesterol-lowering properties [5]. The health benefits of fruit and vegetables are mainly from the phytochemicals and a range of polyphenolics [6]. Significant antioxidant, antitumor, antiviral and antibiotic activities are frequently reported for plant phenols. They have often been identified as active principles of numerous folk herbals. In recent years, the regular intake of fruits and vegetables has been highly recommended, because the plant phenols and polyphenols they contain are thought to play important roles in long term health benefits and reduction in the risk of chronic and degenerative diseases.

2. Synthesis and Structure

Plant secondary metabolites have been fertile area of chemical investigation for many years, driving the development of both analytical chemistry and of new synthetic reactions and methodologies. The subject is multi-disciplinary with chemists, biochemists and plant scientists all contributing to our current understanding [7]. High concentrations of secondary metabolites might result in a more resistant plant. Their production is thought to be costly and reduces plant growth and reproduction [8]. Therefore, defense metabolites can be divided in to constitutive substances, also called prohibitins or phytoanticipins and induced metabolites formed in response to an infection involving de novo enzyme synthesis, known as phytoalexins [9]. Phytoanticipins are high energy and carbon consuming and exhibit fitness cost under natural conditions [10], but recognized as the first line of chemical defense that potential pathogens have to overcome. In contrast, phytoalexin production may take two or three days, as by definition first the enzyme system needs to be synthesized [11].

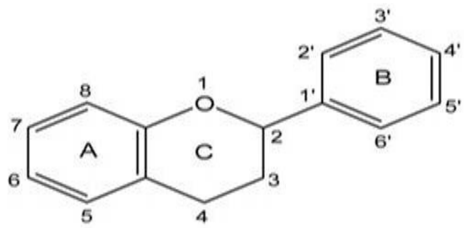
Chemical investigation of plant secondary metabolites remains a fertile area of research from multidisciplinary angles with chemists, biochemists and botanists. Isolation, identification biochemical pathways and contribution of these metabolites in the physiology of plants have enormously enriched the volume of data in last few decades. Based on their biosynthetic origins, plant secondary metabolites are divided into four major groups: (i) terpenoids (ii) N-containing alkaloids (iii) sulfur containing compounds and (iv) phenolics (**Table1**). Phenolics are reported as most widely studied compounds amongst them.

Plant phenolics are synthesized from carbohydrates via shikimate pathway. This is commonly present in plants and microbes as biosynthetic route to aromatic acids. Phenolics are characterized by having at least one aromatic ring with one or more hydroxyl groups attached. In excess of 8000 phenolic structures have been reported and they are widely dispersed throughout the plant kingdom [12]. Phenolics range from simple, low molecular-weight, single aromatic-ringed compounds to large and complex tannins and derived polyphenols. Based on arrangement of their carbon atoms and the number they are commonly found as conjugated to sugars and organic acids. In general, phenolics are distributed into two groups the flavonoids and the non-flavonoids.

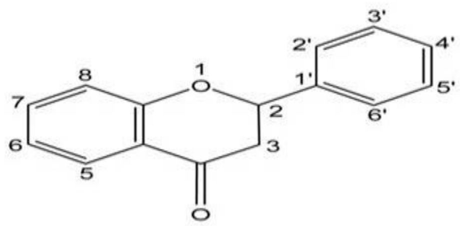
2.1. Flavonoids

Flavonoids are polyphenolic compounds comprising fifteen carbons, with two aromatic rings connected by a three-carbon bridge. They are the most numerous of the phenolics and are found throughout the plant kingdom [13]. They are present in high concentrations in the epidermis of leaves and the skin of fruits and have important and varied roles as secondary metabolites. In plants, flavonoids are involved in such diverse processes as UV protection, pigmentation, stimulation of nitrogen-fixing nodules and disease resistance [14]. The main subclasses of flavonoids are the flavones, flavonols, flavan-3-ols, isoflavones, flavanones and anthocyanidins. Other flavonoid groups, which quantitatively are in comparison minor components of the diet, are dihydroflavonols, flavan-3,4-diols, coumarins, chalcones, dihydrochalcones and aurones.

2.1.1. Basic structure of flavonoid

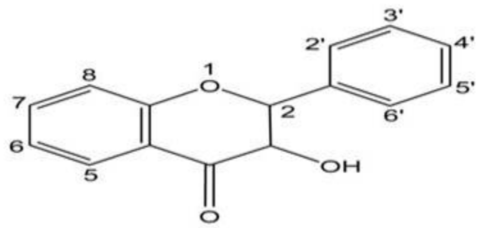


(a) Flavones
(eg. Apigenin, Luteolin, Chrysin)



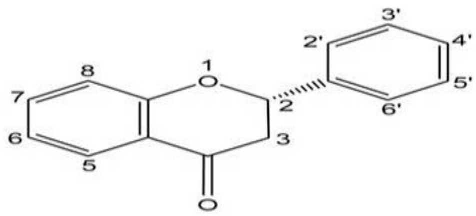
Position	5	7	3’	4’
Apigenin	OH	OH	-	OH
Luteolin	OH	OH	OH	OH
Chrysin	OH	OH	-	-

(b) Flavonols
(eg. Quercetin, Kaempferol, Galangin)



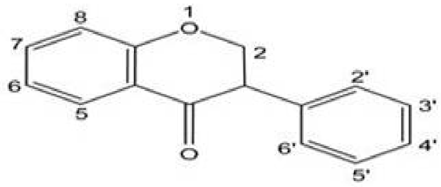
Position	5	7	3’	4’	5’
Quercetin	OH	OH	OH	OH	-
Kaempferol	OH	OH	-	OH	-
Galangin	OH	OH	-	-	-

(c) Flavanone
(eg. Naringenin, Hesperetin)



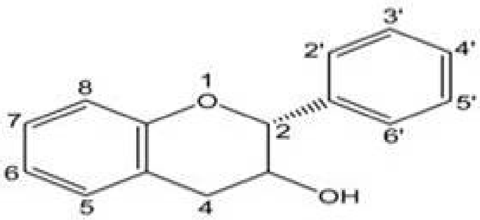
Position	5	7	3’	4’
Naringenin	OH	OH	-	OH
Hesperetin	OH	OH	OH	OCH_3

(d) Isoflavones
(eg. Ganistein, Daidzein)



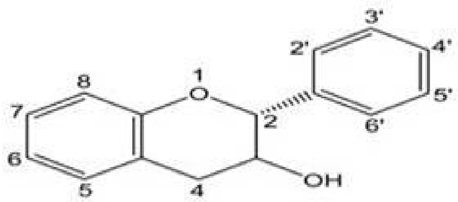
Position	5	7	4’
Ganistein	OH	OH	OH
Daidzein	-	OH	OH

(e) Flavan-3-ol
(eg. (+)- Catechin, (-)-Epicatechin, (-)-Epigallocatechin)



Position	3	5	7	3’	4’	5’
(+)-Catechin	β OH	OH	OH	OH	OH	-
(-)Epicatechin	α OH	OH	OH	OH	OH	-
(-)Epigallocat-echin	α OH	OH	OH	OH	OH	OH

(f) Flavanol
(eg.Taxifolin)



Position	5	7	3’	4’
Taxifolin	OH	OH	OH	OH

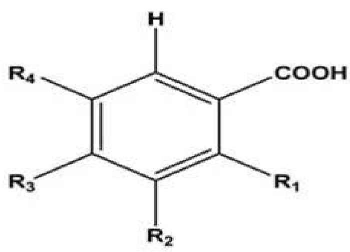
2.2 Non-flavonoids

The main non-flavonoids of dietary significance are the C6–C1 phenolic acids, most notably gallic acid, which is the precursor of hydrolysable tannins, the C6–C3 hydroxycinammates and their conjugated derivatives, and the polyphenolic C6–C2–C6 stilbenes [5].

2.2.1. Phenolic acids

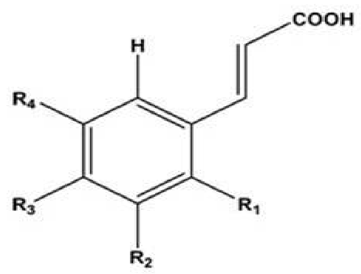
Phenolic acids are also known as hydroxybenzoates, the principal component being gallic acid. The name derives from the French word galle, which means a swelling in the tissue of a plant after an attack by parasitic insects. The swelling is from a build up of carbohydrate and other nutrients that support the growth of the insect larvae. It has been reported that the phenolic composition of the gall consists of up to 70% gallic acid esters [15].

(a) Hydroxybenzoic Acids



Position	R1	R2	R3	R4
Benzoic acid	H	H	H	H
Gallic acid	H	OH	OH	OH
Vanillinic acid	H	OCH_3	OH	H
Salicylic acid	OH	H	H	H

(b) Hydroxycinnamic Acids



Position	R1	R2	R3	R4
Cinnamic acid	H	H	H	H
Ferulic acid	H	OCH_3	OH	H
Sinapic acid	H	OCH_3	OH	OCH_3
Caffeic acid	H	OH	OH	H

Figure 2: Structures of the important naturally occurring phenolic acids (a) Hydroxybenzoic Acids (b) Hydroxycinnamic Acids

2.2.2. Stilbenes

Members of the stilbene family which have the C6–C2–C6 structure, like flavonoids, are polyphenolic compounds. Stilbenes are phytoalexins, compounds produced by plants in response to attack by fungal, bacterial and viral pathogens. Resveratrol is the most common stilbene [16].

The phenolics are present in all parts of the plant, however, quantity differ from one part to other and also with the age of the plant. Quantification data of the same species may also vary with ecophysiological conditions. Thus data on quantification of phenolics are often questioned [17] mainly due to diverse extraction and quantification procedure. Infect, determination of phenolics depends on analytic strategy of the selected sample the analytes and nature of the problem. In general, analysis of phenolics includes separation, identification and measurement using range of solvents and their combinations (**Table 2**). In majority of the methods separation is achieved by HPLC, although GC is used in some instances. HPLC is a versatile and widely used technique for the isolation of natural products. HPLC is a chromatographic technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify and purify the individual components of the mixture mainly because it offers high performance over ambient pressure [18]. For phenolics, RP-HPLC (reverse phase) is most common mode of separation is explored with a C18 column and variable mobile phases (**Table 2**).

Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of herbal plants. The resolving power of HPLC is ideally suited to the rapid processing of such multi component samples on both an analytical and preparative scale [19].

HPLC is a dynamic adsoption process and is a separation technique conducted in the liquid phase in which a sample is separated into its constituent components by distributing between the mobile phase and stationary phase. HPLC utilizes a liquid mobile phase to separate the components of a mixture. The stationary phase can be a liquid or a solid phase. These components are first dissolved in a solvent, and then forced to flow through a chromatographic column under a high pressure [20] .

Reverse-phase chromatography is the most commonly used separation technique in HPLC due to its broad application range. It is estimated that over 65% of all HPLC separations are carried out in the reversed phase mode. The reasons for this include the simplicity, versatility and scope of the reverse-phase method as it is able to handle compounds of a diverse polarity and molecular mass e.g. to identify secondary plant metabolites [21].

In addition, the term used for mobile phases in reversed phase chromatography is “buffer”. However, there is little buffering capacity in the mobile phase solutions since they usually contain strong acids at low pH with large concentrations of organic solvents. Adequate buffering capacity should be maintained when working closer to physiological conditions [22].

In order to identify compound by HPLC a detector must first be selected. Once the detector is selected and is set to optimal detection settings, a separation assay must be developed. UV detectors are popular among all the detectors because they offer high sensitivity and also because majority of naturally occurring compounds encountered have some UV absorbance. Photodiode Array (PDA) and UV-VIS detectors at wavelengths 190-380 nm are normally used to identify the phenolics [21].

The high sensitivity of UV detection is bonus if a compound of interest is only present in small amounts within the sample. Besides UV, other detection methods are also being employed to detect phytochemical among which is the Diode Array Detector (DAD) coupled with Mass Spectrometer(MS) [23].

Liquid chromatography coupled with Mass Spectrometry (LC/MS) is also a powerful technique for the analysis of complex botanical extracts. It provides abundant information for structural elucidation of the compounds when tandem mass spectrometry (MS) is applied. Therefore, the combination of HPLC and MS provide better facilities for rapid and accurate identification of chemical compounds in medicinal herbs, especially when a pure standard is unavailable [24]. HPLC combined with diode array detector (HPLC/DAD), electrochemical detection (HPLC-ED), mass-spectrometer (HPLC/MS) have been successfully employed in qualitative and quantitative determination of various types phytoconstituents including alkaloids, flavonoids, tannins, glycosides, triterpenes, sterols etc [25]. The processing of a crude source material to provide a sample suitable for HPLC analysis as well as the choice of solvent for sample reconstitution can have a significant bearing on the overall success of natural product isolation [26]. The source material, e.g., dried powdered plant, will initially need to be treated in such a way as to ensure that the compound of interest is efficiently liberated into solution. In the case of dried plant material, an organic solvent (e.g., methanol, chloroform) may be used as the initial extracting and following a period of maceration, solid material is then removed by decanting off the extract by filtration [23]. The filtrate is then concentrated and injected into HPLC for separation. The usage of guard columns is necessary in the analysis of crude extract. Many natural product materials contain significant level of strongly binding components, such as chlorophyll and other endogenous materials that may in the long term compromise the performance of analytical columns. Therefore, the guard columns will significantly protect the lifespan of the analytical columns [22]. So, HPLC is a versatile, reproducible chromatographic technique for the estimation of secondary metabolites in the plants. It has wide applications in different fields in term of isolation, quantitative and qualitative estimation of active molecules. In Table-2 an overview of advanced extraction techniques to isolate and purify of plant based compounds, primarily by HPLC technique is summarized.

An antioxidant by definition is a substance that significantly delays or prevents oxidation of its oxidizable substrate when present at low concentrations compared to those of its substrate (Halliwell and Gutteridge 1989; Halliwell 1990). Packer et al. (1995) stated that many criteria must be considered when evaluating the antioxidant potential of a compound. Some of these concerning chemical and biochemical aspects are: specificity of free radical quenching, metal chelating activity, interaction with other antioxidants, and effects on gene expression [27].

Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs, and crude plant drugs. Free radical damages the structural and functional components of the cell such as lipid, protein, carbohydrates, DNA, and RNA. Banana peel contains high content of micronutrient compared to fruit pulp [28]. It attracts great attention because of their nutritional and antioxidant properties, especially the compounds, ascorbate, catechin, gallocatechin, and dopamine. Due to the importance of these compounds, it is necessary to understand its initial production and losses during fruit development, ripening, and maturation [29].

It is well established that phenolic compounds are commonly distributed in plant leaves, flowering tissues and woody parts such as stem and bark. The antioxidant potential of plant materials strongly correlates with their content of the phenolic compounds [30]. In plants, these antioxidant phenolics play a vital role for normal growth and protection against infection and injuries from internal and external sources [31,32].

Different parts of the same plant may synthesize and accumulate different compounds or different amounts of a particular compound due to their differential gene expression, which in turn affects the antioxidant potential and other biological properties of the plant extracts produced [33,34]. Many studies have confirmed that the amounts and composition of phenolic and flavonoid compounds is diversified at the sub-cellular level and within plant tissues as well [35,36]. Plant phenolics, such as phenolic acids, stilbenes, tannins, lignans, and lignin, are especially common in leaves, flowering tissues, and woody parts such as stems and barks [37].

A universally define acceptable solvent, 80 % MeOH and 70 % EtOH are generally preferred solvents for phenolics extraction from plants [38]. The DPPH (2,2-Diphenyl-1-picrylhydrazyl radical) radical is widely utilized to evaluate the free radical scavenging capacity of antioxidants [39]. The DPPH is one of the few stable organic nitrogen radicals, and has a purple color. The radicals absorb at 517 nm. Antioxidant potential can be determined by monitoring the decrease in the absorbance. The result is reported as the amount of antioxidant utilized to decrease the initial DPPH concentration by 50%. The assay is simple and rapid; however, the interpretation is difficult when the test samples have maximum absorption in the range of UV-light that overlaps with DPPH at 517 nm [38].

The phenolic compounds known for its radical scavengers, therefore, it is worthwhile to determine the phenolic content in the plant chosen for the study [40]. Many available methods of quantification of total, mono and di phenolic content in food products or biological samples are based on the reaction of phenolic compounds with a colorimetric reagent, which allows measurement in the visible portion of the spectrum. The monohydroxy benzoic acids act as very weak antioxidants: owing to the electronegative potential of a single carboxyl group, only m-hydroxy bezoic acid has antioxidative potential. This activity increases considerably in the case of dihydroxy substituted benzoic acids, whose antioxidant response is dependent on the relative positions of the hydroxyl groups in the ring. Gallic acid (3,4,5-trihydroxy benzoic acid) is the most potent antioxidant of all hydroxybenzoic acids [41].

Due to the great variety and reactivity of phenolic compounds, the analysis is very challenging [42]. In the early days of high-performance liquid chromatography, it was stated that: “While LC gives accurate, specific results, it is slow relative to total phenol assay procedures, requires expensive equipments and specialized skills. Moreover, in many cases, the details provided by this method (i.e. relative concentrations of each isomer) are not needed”. Even though some of those claims are basically still valid, the introduction of enhanced resolution and increased automation has resulted in HPLC (also known as high-pressure liquid chromatography) becoming the most popular analysis method for plant phenolics [43] .

3. Conclusion

The most studied bioactivity of the phenols is their antioxidant status. The action of phenols as antioxidants is viewed in plants where phenols are oxidized in preference to other food constituents or cellular components and tissues. Thus, measurement of antioxidant potential of a phenol or mixture of phenols has been applied. The need for profiling and identifying individual phenolic compounds has seen traditional methods replaced by high-performance chromatographic analyses. The limited volatility of many phenols has restricted the application of GC to their separation. Merken and Beecher (2000) [44] have presented a comprehensive review on the analytical chemistry of food flavonoids in which they present detailed tabulations of columns and mobile phases used in HPLC. The most common mode of separation exploits reversed-phase systems typically with a C18 column and various mobile phases.

Table 1: Number of Secondary Metabolites reported from higher plant (Satyawati and Gupta 1987)

Type of secondary metabolite	**Approximate numbers**
Nitrogen-containing Secondary metabolites	
Alkaloids	21000
Amines	100
Non-protein amino acids (NPAAS)	700
Cyanogenic glycoside	60
Glucosinolates	100
Alkamides	150
Lectins,peptides,polypeptide	2000
Secondary metabolites without nitrogen	
Monoterpenes including iridoids	2500
Sesquiterpenes	5000
Diterpenes	2500
Triterpenes, steroids, saponins	5000
Tetraterpenes	500
Flavonoids, tannins	5000
Phenylpropanoids, lignin, coumarins, lignans	2000
Polyacetylenes, fatty acid, waxes	1500
Anthraquinones and othes polyketides	750
Carbohydrates, organic acids	200

Table 2: Review on various parameters of phenolic compounds investigated in plants using HPLC analysis.1

Sr No.	Plant name	Plant family	Plant part	HPLC System				Use of the compound
				Column	Mobile phase	Compound extracted		
						Phenols	Flavanoids	
1	*Alpinia officinarum*	Zingiberaceae [46]	air dried leaves powder	A separon SIX C18 (5mm) RP-cartridge 15cm ´3 mm I.D.	methanol- 5mM diammonium hydrogenphosphate pH 7.3 (65:35)	Vinblastin		use in neoplastic diseases
2	*Betula pubescens*	Betulaceae; [47]	leaves	Spherisorb ODS-2 Col.(250×4.6mm i.d.5µm)	A.5% aq.Formic acid B.Acetonitrile	2 acylated compounds (1st time) 1. myricetin-3-O-α-L-(acetyl)-rhamnopyranoside 2. quecetin-3-O-L-(4-O-acetyl)-rhamnopyranoside Chlorogenic acid	Myricetin glycosides Quecetin Glycosides Kaempferol glycosides	Antioxidant activity
3	*Camellia sinensis* L.	Theaceae [48]	dry leaves	2 type column 1. Nova Pak C18-4mm column (3.9mm´15 cm) from waters (miliford MA)	Acetonitrile, ethyl acetate, methanol in combination	Catechins, caffeine		antioxidant, anti-mutagenic, anti-carcinogenic, hypochol-esterolemic activity
				2.Ultrapac Spherisorb ODS 2-3 mm column (4.6mm´10 cm) from LKB (Bromma, Sweden) UV detector	with 0.1% orthophossphoric acid 8.5:2:89.5,v/v/v phase			
4	*Melissa officinalis* L.	Lamiaceae [49]	dried leaves	Lichrocart 125-4 superspher RP 8-E, 4mm (Merck)	A.H_2O-H_3PO_4 85% (100:0.3)	Luteolin derivatives		
					B.MeCN-H2O-H3PO4 85% (80:20:0.3)	Rosmarinic acid		and functoinal gastrointestinal disorders
5	*solanum nigrum*	Solanaceae [50]	root,stem, leaves	ODS-col. 25´0.26 cm	1.CAN 2. 0.01M tris	solasonine, solamargine, solanine (glycoalkaloids)		pharmaceutical industry
6	*Schisandra chinensis Baill.*	Schisandraceae [51]	seeds	separon SGX C18 5mm (150 ´ 3 mm I.D)	methanol- deionised water (75:25)	lignin separation Gomisin A, Gomisin B		prevent liver injuries, lipid peroxidation
		Mangnoliaceae						stimulate liver regeneration
								inhibit hepato-carcinogenesis
7	*Lactuca sativa L.*	Asteraceae [52]	leaves	150´3 mm(5 mm) Luna C18 col. With 4 mm ´ 3 mm I.D. C18 ODS precol.	4 step linear gradient system used starting from 93% water (pH 3.2 by H_3PO_4) upto 75% CH_3CN	caffeic acid, chlorogenic acid isochlorogenic acid polyphenols		treatment of rhinitis, asthma, cough and pertussis
8	*Beet roots*	Amaranthaceae [53]	roots	1. a Li- chrospher 100RP-18 125 ´ 4 mm, 5mm with guard col. 4´ 4mm, 5mm 2. a zorbax SB C8 150´ 4.6 mm, 5 mm guard col.12.5 ´4.6 mm, 5mm	binary gradient mixture of 2. 30mM potassium phosphate buffer at pH 2.3 and acetonitrile	Folates (naturally occuring vitamin B)		health protecting roles

9	*Hamamelis virginiana L* Witch hazel.	Hamamelidaceae [54]	dry twing, bark, leaves	A kingsorb 5mm C18 (150 ´ 4.6mm)	A.0.1% (v/v) orthophosphoric acid in water B. 0.1% (v/v) orthophosphoric acid in methanol	Hamamelitanin catechins gallic acid		used as components of skincare products. in dermatological treatment of sunburn, irritated skin, atopic eczema. to promote wound healing via anti-inflammtory effects
10	*Centaurium erythraea*	Gentianaceae [55]	Micropropagate plant	hypersil ODS col. (250 ´ 4 mm 5mm hewlett packard	1.ACN 2.3% v/v acetic acid	secoiridoid glucosides gentiopioside, sweroside, swertiamarine		fungitoxic, antibacterial, choleretic, pancreatic, hepato-protective
11	*Alpinia officinarum Hance(AO)*	Zingiberaceae [56]		RP-col.(ZOR BAX, Eclipse SB- C18 5mm, 4.6 ´ 250 mm) C18 guard col.	methanol-water-phosphoric acid(60-38-2,v/v/v isocratically		Flavonoid Galangin 3-O-methayl galanin	anticlastogenic, anti-mutagenic, anti-oxidative, radical scavenging hypolipidemic agent due to its inhibition of pancreatic lipase
12	*Piper regnellii (Miq.) C.DC. Var*	Piperaceae [57]	dried root stem leaves	Metasil ODS col. 5mm 150mm ´4.6 mm	mixture of acetonitrile-water(60:40 v/v)	conocarpan (neolignan)		use for treatment of wounds, swellings, skin irritations
	Pallescens (C.DC.) Yunck				containing 2% acetic acid	eupomatenoid-5 eupomatenoid-6		
13	*Platycladus orientalis L.*	Cupressaceae [58]	leaves	Agilent Eclipse XDB-C18 Col. (3.5mm,12.5mm´4.6 mm I.D.)	methanol and CAN		flavanoids	antioxidant, antiallergic diuretic properties
	Franco						Quercitrin amentoflavone	use for treatment of gout, rheumatism, diarrhea
14	*Paeonia lactiflora* oriental medicinal plant	Paeoniaceae [59]	dry plant	Inertsil ODS-3 C18 (250 ×6mm) 5mm I.D C18 guard col.	acetonitrile- water (gradient HPLC method	Paeoniflorin, albiflorin		cleansing heat, cooling blood, invigoraing blood circulation
15	*Prunus x domestica* L.	Rosaceae [60]	fruit, leaf, leaf petiole	SGE Walkosil 11 5C18 RS column (150 ´ 4.6 i.d.5mm particles	50 mM NaC_{l4} in 0.1% H_3Po_4	Ghrelin hormone		promotes food intake, weight gain and adiposity in rodents
	Marus alba	Moraceae		120 A Pore size		(in Parenchyma cells)		
16	*Vitex-agnus-castus*	Verbenaceae [61]	dry leaves	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	Caffeic acid		Antioxidant activity
					B. water with 6% glacial acetic acid	Ferulic acid		
					C. water acetonitril(65:30 v/v) with 5% glacial acetic acid	Rutin, p-Coumaric acid		
17	*Origanum dictamnus*	Lamiaceae [61]	dry leaves	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	Catechin		Antioxidant activity
					B. water with 6% glacial acetic acid			
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid			

18	*Teucrium polium*	Lamiaceae [61]	dry leaves	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	Tyrosol		Antioxidant activity
					B. water with 6% glacial acetic acid	Caffeic acid		
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid	Ferulic acid, luteolin		
19	*Lavandula vera*	Lamiaceae [61]	dry leaves	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	p-hydroxybenzoic acid		Antioxidant activity
					B. water with 6% glacial acetic acid	Catechin		
					C. water acetonitril(65:30 v/v) with 5% glacial acetic acid	Vanillic acid Caffeic acid, Ferulic acid, Naringenin		
20	*Lippia triphylla*	Verbenaceae [61]	dry leaves	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	Hydroxytyrosol		Antioxidant activity
					B. water with 6% glacial acetic acid	Caffeic acid		
					C. water acetonitril (5:30 v/v)with 5% glacial acetic acid	Ferulic acid, Apigenin		
	Greek aromatic olive plants							
21	*Capparis spinosa*	Capparaceae [61]	leaves	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	Hydroxytyrosol	Quercetin	significance for human diet and antimicrobial activity
					B. water with 6% glacial acetic acid	Caffeic acid	Rutin	
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid	p-Coumaric acid, Vanillic acid, Ferulic acid, Gallic acid		
22	*Castanea vulgaris*	Cupuliferae [61]	leaves	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	gallic acid	quercetin	significance for human diet and antimicrobial activity
					B. water with 6% glacial acetic acid	ferulic acid	rutin	
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid		naringenin	
23	*Geranium purpureum*	Geraniaceae [61]	leaves	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	gallic acid	quercetin	significance for human diet and antimicrobial activity
					B. water with 6% glacial acetic acid	gentisic acid	rutin	
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid	caffeic acid, p-Coumaric acid, vanillic acid, syringic acid, p-hydroxybenzoic acid		
24	*Nepeta cataria*	Labiateae [61]	herb	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	gallic acid	luteolin	significance for human diet and antimicrobial activity
					B. water with 6% glacial acetic acid	caffeic acid	eriodictyol	
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid	ferulic acid		
25	*Origanum dictamnus*	Labiateae [61]	leaves	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	gallic acid	quercetin	significance for human diet and antimicrobial activity

					B. water with 6% glacial acetic acid	caffeic acid		
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid	p-Coumaric acid, p-hydroxybenzoic acid		
26	*Spartium junceum*	Leguminosae [61]	flower	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	gentisic acid	luteolin	significance for human diet and antimicrobial activity
					B. water with 6% glacial acetic acid	caffeic acid	quercetin	
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid	p-Coumaric acid, vanillic acid		
27	*Jasminum officinalis*	Oleaceae [61]	flower	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	gallic acid	quercetin	significance for human diet and antimicrobial activity
					B. water with 6% glacial acetic acid	p-Coumaric acid		
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid			
28	*Phytolacca americana*	Phytolaccaceae [61]	leaves	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	caffeic acid	rutin	significance for human diet and antimicrobial activity
					B. water with 6% glacial acetic acid	p-Coumaric acid		
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid	vanillic acid, p-hydroxybenzoic acid, ferulic acid		
29	*Ruta graveolens*	Rutaceae [61]	leaves	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid B. water with 6% glacial acetic acid	ferulic acid, p-hydroxybenzoic acid, gentisic acid, caffeic acid	Quercetin, rutin	significance for human diet and antimicrobial activity
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid			
30	*Styrax officinalis*	Styracaceae [61]	leaves	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	gallic acid	quercetin	significance for human diet and antimicrobial activity
					B. water with 6% glacial acetic acid	gentisic acid	naringenin	
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid	caffeic acid, p-Coumaric acid, vanillic acid, p-hydroxybenzoic acid, ferulic acid		
31	*Cuminum cymium*	Umbelliferae [61]	seeds	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	gallic acid	rutin	significance for human diet and antimicrobial activity
					B. water with 6% glacial acetic acid	caffeic acid	quercetin	
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid	ferulic acid	naringenin	
32	*Foeniculum vulgare*	Umbelliferae [61]	herb	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	gallic acid	rutin	significance for human diet and antimicrobial activity
					B. water with 6% glacial acetic acid	caffeic acid	quercetin	
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid	ferulic acid		

33	*Himulus hipulus*	Urticaceae [61]	leaves	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	gallic acid	eriodictyol	significance for human diet and antimicrobial activity
					B. water with 6% glacial acetic acid	p-Coumaric acid	quercetin	
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid	p-hydroxybenzoic acid		
34	*Urtica dioica*	Urticaceae [61]	leaves	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid	gallic acid		significance for human diet and antimicrobial activity
					B. water with 6% glacial acetic acid	syringic acid		
					C. water acetonitril (65:30 v/v) with 5% glacial acetic acid	ferulic acid		
35	*Canarium album* L.	Burseraceae [62]	fruit	RP-C18(250mm´4mm)	A.0.5% (v/v) acetic acid	Gallic acid	1st reported	used for the treatment of faucitis, stomatitis, hepatitis, toxicosis
	chinese olive fruit		budding to flower stage		A. methanol	methyl gallate, ethay gallate, ellagic acid, brevifolin carboxylic acid, sinapic acid, hyperin		
					B. water with 0.2% sulphuric acid	rosmarinic acid, vanillic acid, chlorogenic acid, gallic acid, cinnamic acid	Luteolin, coumarin, rutin	strong antioxidant activity, oil as antimicrobial agent
36	*Tobacco*	Solanaceae [63]	dried leaves	Hypersil C4 (4.6mm ´ 150 mm,5mm	acetonitrile and water	solenosol		cardiac stimulant lipid antioxidant antibiotic
37	*Echinacea pallida* (Nutt)	Asteraceae [64]	dry root/capsules	1.as above 2. chromolith performance RP-C18 (100mm´4.6mm guard col. RP-C18 (5mm´4.6mm)	1.water 2.ACN	Polyacetylenes polyenes		antifungal, antibacterial compound inhibitor of no. of enzymes eg. Cholesterol, acytransferase
38	*Borago officinalis L.*	Boraginaceae [65]	leaves	TOSO HAAS Semi-micro ODS-80 TS col. (5mm,2mm´ 25 cm)	A. 2% acetic acid B. acetonitrile	rosmarinic acid		antioxidant and antiradical activity
39	*Alpinia zerumbet*	Zingiberaceae [66]	leaves, roots	RP-18 ZORBAX ODS col. 25 ´ 0.46 cm 5mm particle size	A.1% v/v acetic acid B. methanol/ acetonitrile/ acetic acid (95:4:1 v/v/v)	from leaves oil 1,8 cineol, methyl cinnamate	Rhizomes DDK, methyl cinnamate, dihydro-5,6-dehydrokawain	insecticidal antifungal activity
40	*Alpinia zerumbet*	Zingiberaceae [66]	flower, seeds	RP-18 ZORBAX ODS col. 25 ´ 0.46 cm		Flower	seeds	antioxidant activity
				5mm particle size	A.1% v/v acetic acid B. methanol/ acetonitrile/ acetic acid (95:4:1 v/v/v)	syringic acid, p-hydroxybenzoic acid, ferulic acid	p-hydroxybenzoic acid, vanillic acid, syringic acid	used for the treatment of cardiovascular hypertensions, antipasmodic agent
41	*Anisophyllea dichostyla R.Br.*	Rhizophoraceae [67]		C18-RP col. (250´4mm 5mm)	1.2% acetic acid 2.methanol	catechins ellagic acid derivatives epicatechin		medication against anorexia, fatigue and intestinal infection

42	*Hippophae rhamnoids*	Elaeagnanceae [68]	SB berriers, leaves	phenomenex c18,ODS-2,5mm, 250mm´4.6mm	A.2% acetic acid B.metanol	protocatechuic acid, p-hydroxybenzoic acid, cinnamic acid, vanillic acid, gallic acid,		antitumor, antiviral, antioxidant properties, medicinal and cosmetic applications
	Sea Buckthorn					caffeic acid, p-Coumaric acid, ferulic acid, salicyclic acid		
43	*Vitis vinifera* L. *(white)*	Vitaceae [69]	Grapes	C18 kromasil 300mm´4.6mm 5mm particle size	A.acetonitrile/ acetic acid/ water(35:2:63) B. 2% acetic acid	5 DHF (dihydroflavonols) 7Q (quercetin derivatives), 4 Kaempferol derivatives		produce high quality wine
44	Cymbopogon citrates *lemon grass*	Gramineae [70]	leaves	Spherisorb.S5 ODS-2 column (250 ´4.6mm I.D 5mm) guard column C18 (30 ´ 4 mm I.D 5mm)	A. 5% aqueous formic acid v/v B. methanol	O and C glycosyl flavones Apigenin, loteolin		anti inflammatory, diuretic activities Hypotensive, vasorelaxating
45	*Rosmarinus officinalis*	Labiatae [71]	Oil	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid B. water with 6% glacial acetic acid C. water acetonitril(65:30 v/v)with 5% glacial acetic acid	syringic acid ferulic acid		multiple biological effect such as antioxidant activity antimicrobial activity prevention of human pathologies
46	*Origanum dictamnus*	Labiatae [71]	oil	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid B. water with 6% glacial acetic acid C. water acetonitril(65:30 v/v)with 5% glacial acetic acid	caffeic acid	Naringenin, eriodictyol	multiple biological effect such as antioxidant activity antimicrobial activity prevention of human pathologies
47	*Origanum majorana*	Labiatae [71]	oil	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid B. water with 6% glacial acetic acid C. water acetonitril (65:30 v/v) with 5% glacial acetic acid	high ferulic acid	Catechin, rutin, quercetin	multiple biological effect such as antioxidant activity antimicrobial activity prevention of human pathologies
48	*vitex-agnus-cactus*	Verbenaceae [71]	oil	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid B. water with 6% glacial acetic acid C. water acetonitril (65:30 v/v) with 5% glacial acetic acid	P-hydroxybenzoic acid		multiple biological effect such as antioxidant activity prevention of human pathologies
49	*styrax officinalis*	Styracaceae [71]	oil	5mm ODS 2.4´250 mm at ambient temp.	A. water with 1% glacial acetic acid B. water with 6% glacial acetic acid C. water acetonitril (65:30 v/v) with 5% glacial acetic acid	vanillic acid		multiple biological effect such as antioxidant activity antimicrobial activity prevention of human pathologies

50	*Ephedra sinica stapfs*	Ephedraceae [72]		HS F5 col. (150mm ´ 4.6 mm I.D.5mm	Isocratic ammonium acetate (7mM) in acetonitrile-water (90:10v/v)	ephedrin alkaloids		for treatment of asthma, bronchial spams, as a stimulant and diaphoretic
	Ephedra vulgaris Rich.				ammonium acetate (7mM) in acetonitrile-water (90:10v/v)	Synephrine Norephedrine Norpseudoephedrine Ephedrine		Used as a as a stimulant and diaphoretics
51	*Eucommia ulmodies Oliver.*	Eucommiaceae [73]	dried leaves	C18 col.(150mm ´ 4.6 mm I.D.5mm)	1. methanol 2. 0.5% acetic acid	chlorogenic acid		antibacterial, antimutagenic, antioxidant, quality control
52	*Eugenia jambolana Lam.*	Myrtaceae [74]	bark	ODS RP C18 col.(250 ´4.6 mm, 5mm	1. 3% trifluoroacetic acid 2. acetonitrile-methanol (80:20 v/v)		Quercetin Kampeferol total flavonols	antioxidant, antiallergic, anti-artherogenic, anti-inflammatory, antimicrobial antihrombotic, cardio-protective, vasodilatory effect
53	*Acacia nilotica*	Fabaceae [74]	bark	ODS RP C18 col.(250 ´4.6 mm, 5mm	1. 3% trifluoroacetic acid 2. acetonitrile-methanol (80:20 v/v)		Myricetin Quercetin Kampeferol total flavonols	antimicrobial antihrombotic, cardio-protective, vasodilatory effect
54	*Azadirachta indica*	Meliaceae [74]	bark	ODS RP C18 col.(250 ´4.6 mm, 5mm	1. 3% trifluoroacetic acid 2. acetonitrile-methanol(80:20 v/v)		Quercetin Kampeferol total flavonols	antimicrobial antihrombotic, cardio-protective, vasodilatory effect
55	*Terminalia arjuna*	Combretaceae [74]	bark	ODS RP C18 col.(250 ´4.6 mm, 5mm	1. 3% trifluoroacetic acid 2. acetonitrile-methanol (80:20 v/v)		Quercetin total flavonols kampeferol	antimicrobial antihrombotic, cardio-protective, vasodilatory effect
56	*Moringa oleifera*	Moringaceae [74]	leaves, roots	ODS RP C18 col.(250 ´4.6 mm, 5mm	1. 3% trifluoroacetic acid 2. acetonitrile-methanol (80:20 v/v)		**Leaves** Myricetin Quercetin Kampeferol total flavonols	antimicrobial antihrombotic, cardio-protective, vasodilatory effect
							Roots Myricetin Kampeferol total flavonols	antimicrobial antihrombotic, cardio-protective, vasodilatory effect
57	*Ficus religiosa*	Moraceae [74]	fruit	ODS RP C18 col.(250 ´4.6 mm, 5mm	1. 3% trifluoroacetic acid 2. acetonitrile-methanol (80:20 v/v)		Myricetin Kampeferol Quercetin total flavonols	antimicrobial antihrombotic, cardio-protective, vasodilatory effect
58	*Aloe barbadensis*	Asphodelaceae [74]	leaves	ODS RP C18 col.(250 ´4.6 mm, 5mm	1. 3% trifluoroacetic acid 2. acetonitrile-methanol (80:20 v/v)		Myricetin Kampeferol Quercetin total flavonols	antimicrobial antihrombotic, cardio-protective, vasodilatory effect

59	*Pinguicula lusitanica* L. Pale butter wort carnivorous perennial plant	Lentibulariaceae [75]	invitro plantlets used	An alltima HP 3mm (150 ´ 4.6 mm I.D.)	A. 0.1% v/v formic acid B. acetonitrile	iridoid glucosides, Caffeoyl Phenylethanoid Glycosides a. globularin b. verbascoside		imp role in defense mechanism against herbivores. resistance to or protection from fungal and viral attacks
60	*Viburnum prunifolium*	Caprifoliaceae [76]		A Pinnacle ODS amine C18 (250mm´4.6mm, 5mm protected by	As above	iridoidic component		
	Black Haw			ODS amine C18 guard col. (10mm ´4 mm,5mm)		iridoid glucosides		
61	*Glycine max L.*	Fabaceae [77]	seed	RP-C18 col. (125mm ´ 4mm, Lichro) (ART,5mm Merck K GaA)	1. 0.1% acetic acid in water	9 type anthocyanin		Detoxification
	black soyabean				2. 0.1 % acetic acid in CAN	1. catechin-cyanidin-3-O-glucoside 2. delphinidin-3-O-galactoside 3. delphinidin-3-O-glucoside 4. cyanidin-3-O-Galactoside 5. cyanidin-3-O-glucoside		anti inflammatory
						6. petunidin-3-O-Glucoside 7. pelargonidin-3-O-glucoside 8. peonidin-3-O-glucoside 9.cyanidin		to improve blood process
62	*Cannabis sativa L.*	Cannabinaceae [78]		waters Xterra MS C18 analytical col. (5mm,250mm ´ 2.1mm)	mixture of methanol/ water containing 50mM of	Cannabinoids, D9 Tetrahydrocannabinol (THC), THC acid (THCA), cannabidiol (CBD)		psychoactive properties
				(5mm 10mm ´ 2.1mm guard col.	ammonium formate	CBD acid (CBDA), cannabigerol (CBG), CBG acid (CBGA), cannabinol (CBN), D8-tetrahydro cannabinol(8- THC)		
63	*Origanum majorana*	Lamiaceae [79]	dried aerial part	250mm´4.6mm, 4mm Hypersil ODS C18 column	A.Acetonitrile	trans-2 hydroxy cinnamic acid	Amentoflavone, apigenin, quercetin	used as fungicides and insecticides
			budding to flower stage		B.water with 0.2% sulphuric acid	rosmarinic acid, vanillic acid, chlorogenic acid, gallic acid, cinnamic acid	Luteolin, coumarin, rutin	strong antioxidant activity, oil as antimicrobial agent
64	*Microula sikkimensis*	microula benth family [80]	dried seeds	Eclipse XDB C8 col. (150mm ´4.6mm, 5mm)	A. 50% of acetonitrile	fatty acids		imp in treatment for cardiovascular and hepatic disease
	rare wild oil plant				B. 50% of acetonitrile containing 20mM/L	linolenic acid		
					ammonium formate buffer (pH 3.7)	linolic acid		
					C. mixture solution of acetonitrile and DMF	saturated and unsatutated acid		

					(acetonitrile/ DMF, 100:2 v/v)			
					(acetonitrile/ DMF, 100:30 v/v)			
65	*Rheum emodi*	Polygonaceae [81]	rhizomes	A Purospher- star RP-18e colu.	1.ACN-methanol (95:5 v/v)	Anthraquinone derivatives	1 to 3 6 month TC plant	antifungal, antimicrobial, cytotoxic, antioxidant activities
			tissue culture plant use	4.6mm i.d.´250 mm , 5mm	2. water- acetic acid (99.9-0.1 v/v)	1. emodin glycoside 2. chrysophanol glycoside 3. emodin 4.chrysophanol 5.physcion	9 month TC plant	
66	*Cordia americana*	Boraginaceae [82]	leaves	RP-C18 (5mm´100 mm:5mm)	A.water- acetonitrile: formic acid (90:10:0.1)			anti-inflammatory, wound healing activities
					B.Acetonitrile-formic acid (0.1%)			
67	Allium sativum	Liliaceae [83]	root, shoot, bulbs, leaves	C18 Nucleosil 100 ODS (5mm), analytical col.4.6mm ´ 150mm	methanol- water (50:50 v/v)	allicin		anti diabetic activity IN VITRO antimicrobial, anto-thrombotic, anticancer, antioxidant
	green garlic plant (immature)			C18 guard col.with 20ml loop.				IN VIVO cardiovascular disorders, arteriosclerosis
68	*Rheum emodi*	Polygonaceae [83]	rhizomes	A Purospher- star RP-18e colu.	1.ACN-methanol (95:5 v/v)	Anthraquinone derivatives	1 to 3 6 month TC plant	antifungal, antimicrobial, cytotoxic, antioxidant activities
			tissue culture plant use	4.6mm i.d.´250 mm , 5mm	2. water- acetic acid (99.9-0.1 v/v)	1. emodin glycoside 2. chrysophanol glycoside 3. emodin 4.chrysophanol 5.physcion	9 month TC plant	
69	*Aristolochia species*	Aristolochiales [84]		X Terra MS C18 (150 mm´ 2.1 mm, I.D.5mm	0.2% formic acid water and acetonitrile	Aristolochic acids (Aas)		anti inflammatory agents for arthritis, gout, rheumatism and dieresis
	1. Radix aristolochia					Aristoloctams(Als)		
	2. Caulis aristolochia anshurinensis							
	3.Fructus aristolochia							
70	*Banisteriopsis caapi.*	Malpighiaceae [85]	fresh leaves, stem	Gemini C18 110A° col.	for catechin analysis 1. water 2. acetonitrile	Harmine Harmaline tetrahydro harmine		responsible for mono-aminooxidase (Mao)A inhibitor

			large branch	Phenomax, 150mm ´4.6mm I.D.5mm	for alkaloid analysis 1.acetonitrile containing 0.1% acetic acid 2. 50mM ammonium acetate (pH 4.2)	proanthocynidines like epicatechin		procynidine produce antioxidant effect
71	*C. annuum* L.	Solanaceae [86]	Ripe paprika	C18 Phenomenex column (Torrance, CA, USA) Gemini series (250 ×4.6 mm i.d., 5 lm particle size)	A .(0.03 M phosphoric acid in water) and B. (MeOH)		Quercetin Luteolin Kaempferol	High antioxidant and anticancer activities
72	*Eucommia ulmoides* Oliv.	Eucommiaceae [87]	Dried leaves	reversed phase SunFire™ C18 (250 mm _ 4.6 mm i.d., 5 lm, Milford, MA, USA) column.	A (0.4% acetic acid in water) and B (acetonitrile),	1, geniposidic acid; compound 2, caffeic acid; compound 3; chlorogenic acid; compound 4, ferulic acid; compound 5, quercetin-3-O-sambubioside; compound 6, rutin; compound 7, isoquercitrin.		antioxidant activity (Cho et al., 2003; Yen & Hsieh, 1998), glycation inhibitory activity (Kim, Moon, Lee, & Choi, 2004) and anti-obesity activity
73	Artichoke (*Cynara scolymus* L.)	Asteraceae 88]	Fresh artichoke samples (hearts)	Agilent Zorbax C18 column (4.6 _ 150 mm, 1.8 lm)	A. Acidified water (0.5% acetic acid, v/v) and B. acetonitrile	3 hydroxybenzoic acids, 17 hydroxycinnamic acids, 4 lignans, 7 flavones, 2 flavonols, and 1 phenol derivative		antioxidative, anti-carcinogenic, antigenotoxic, cholesterol-lowering, hepato-protective, bileexpelling, diuretic, and anti-inflammatory, as well as antifungal, anti-HIV, and antibacterial
74	sarang semut (*Myrmecodia pendan*).	Rubiaceae [89]	powder	Luna 5U-C18 (2) 100A column (250 mm ×4.5 mm, 5 _m) plus Jasco, quaternary gradient pump (pu-2089) plus Jasco	A. deionized water and 1% acetic acid B. methanol (HPLC grade) and 1% acetic acid		kaempferol, luteoline, rutine, quercetine and apigenin)	antioxidant activities (Tian et al., 2009), metal chelation (Heim et al., 2002; Seyoum et al., 2006) and anti-proliferative, anti-carcinogenic, antibacterial, anti-inflammatory, antialergic, and antiviral effects
75	*Convolvulus pluricaulis* Shankhpushpi	Convolvulaceae [90]	leaves	Phenomenex C18 column (250 mm × 4.6 mm, 5 µm) (California, USA)	a isocratic mixture of methanol and water containing 0.1% v/v formic acid in the ration of 30: 70.	scopoletin		to treat chronic bronchitis and asthma.
76	*Glycyrrhiza glabra* Linn.	Fabaceae [91]	roots	C-18 reverse phase column (250 x 4.6 mm internal diameter, particle size 5 µm, Luna 5 µm C-18),	methanol: water (70:30 v/v)	important metals like Ca, K, Fe and Mg		Antimicrobial activity
77	water watercress (*Nasturtium officinale*–	Brassicaceae [92]	Dried material	RP-C18 column (4.6 mm × 250 mm) packed with 5-µm diameter particles	methanol-acetonitrile-water (40:15:45, v/v/v) containing 1.0% of acetic acid	Rutin, chlorogenic, and caffeic acids		Antioxidant activity

78	rose hip (Rosa L.) *Rosa canina, Rosa dumalis, Rosa gallica, Rosa dumalis* subsp.boissieri and *Rosa hirtissima*	Rosaceae [93]	Fruits	C18 (250 × 4.6 mm I.D.)	(A) water/acetic acid (98:2) and (B) water/ acetonitrile/acetic acid(78:20:2).	gallic acid, 4-hydroxy benzoic acid, caftaric acid, 2,5-dihidroxy benzoic acid, chlorogenic acid, t-caffeic acid, p-coumaric acid and ferrulic acid	methyl gallat, (þ)-catechin and (_)-epicatechin	Strong antioxidant activities
79	Emblica officinalis	Phyllanthaceae [94]	fruit	A reversed-phase column, Zorbax SB RP C-18 (250mm_4.6mm_5 mm pore size),	0.1% orthophosphoric acid in water (v=v) and acetonitrile	vitamin C (ascorbic acid), phenolic acids (gallic acid and ellagic acid), hydroxycinnamic acid (chlorogenic acid)	myricetin, quercetin, and kaempferol	Use for cancer, cardio-vascular disorders, ageing, diabetes, and Hypertension
80	Swertia chirayita *S. minor, S. densifolia, S. lawii, S. corymbosa and S. angustifolia var.* *pulchella*	Gentianaceae [95]	Powder of whole dry plant	C18e (5 mm) column (250–4.6 mm).	methanol and water (90:10)	BA, betulinic acid; OA, oleanolic acid; UA, ursolic acid		chronic fever, malaria, anaemia, bronchial asthma, liver disorders, hepatitis, gastritis, constipation, dyspepsia
81	*Annona muricata*	Annonaceae [96]	Dried leaf powder	a Waters Symmetry® C18 column (5 mm, 4.6×50 mm) with Waters Sentry TM universal guard column (5 mm, 4.6×20 mm)	A (50 mM sodium phosphate in 10 % methanol; pH 3.3) and B (70 % methanol)	Cinnamic acids (-) –Epicatechin gallate Coumarid acid Anthraquinones Isoferulic acid	Quercetin Luteolin	anti-spasmodoic, hypotensive
82	*Schinopsis brasiliensis* Engl.,	Anacardiaceae [97]	Stem bark	a Phenomenex Gemini NX C18 column (250 × 4.6 mm, 5 _m).	0.05% orthophosphoric acid: methanol	Gallic acid		uses for the treatment of diar-rhea and coughs, and can also be used as an antiseptic and analgesic
83	*Ziziphus joazeiro*	Rhamnaceae [98]	leaves	HPLC–DAD) a Phenomenex C18 column (4.6 mm _ 250 mm) packed with 5-lm diameter particles	A.water containing 1% formic acid and B.acetonitrile	gallic acid, caffeic acid, ellagic acid, catechin and epicatechin	quercetin, isoquercetin, quercitin, kaempferol and rutin	antifungal (Cruz et al., 2007), gastro-protective (Romão, Costa, Terra, Boriollo, & Soares, 2010) and anti-microbial properties (Silva et al., 2011).
84	*Corylus maxima* Mill.	Betulaceae [99]	leaves	a Zorbax SB C18 col-umn (150 mm × 3.0 mm, 3.5 _m;	0.2% (v/v) acetic acid, methanol	myricetin-3-O-rhamnoside and quercetin-3-O-rhamnoside – and two diarylheptanoids – oregonin and hirsutenone		Antioxidant activity
85	*Paronychia argentea* Lam.,	Caryophyllaceae [100]	Aerial part	HPLC-UV/DAD conditions and HPLC–ESI-MSn conditions an Ascentis C18column (250 mm × 4.6 mm I.D.,5 µm,	HPLC-UV/DAD conditions (A) 0.1 M HCOOH in H2O and (B) ACN		isorhamnetin-3-O-dihexoside, quercetin-3-O-glucoside, quercetin methylether-O-hexoside, quercetin, jaceosidin and isorhamnetin (1st time)	Antioxidant activity
86	Libyan herb species, *viz Sage, Thymus,* *Rosemary, Chamomile, Artemisia*	Lamiaceae [101]		C18 reversed-phase analytical column, 5 µm particle size, with dimension 250 × 4.6 mm	A.Buffer solution B.methnol	Rutin Ascorbic acid		antibacterial, anti inflammatory, antitumor, antiallergic, antiviral and antiprotozoal.

87	*Rheum spiciforme* & *Rheum webbianum*	Polygonaceae [102]	Root and rhizomes	C18 column (250 mm× 4.6 mm; Sunfire)	A.methanol B.2% acetic acid	Emodin Aloe Emodin Rhein		anti-cancer and anti-oxidant activities.
88	*Limonium brasiliense* (Boiss.) Kuntze,	Plumbaginaceae [103]	rhizome	an Agilent Zorbax C-18 (250 mm × 4.6 mm) 5 _m column	A.water: concentrated phosphoric acid (100:0.2, v/v,) and B.acetonitrile: concentrated phosphoric acid (100:0.2, v/v,)	GC, gallocathechin; EGC, pigallocatechin.		Anticancer and antioxidant activity
89	*H. perforatum* (St. John's wort), *L. angustifolia* (lavender), *M. sylvestris* (tall mallow), *M. officinalis* (lemon balm), *S. officinalis* (sage) and *R. officinalis* (rosemary)	Lamiaceae [104]	leaves	LiChrospher_ 100, RP-18 (250 _ 4 mm, 5 lm) column,	A (methanol), B (acetonitrile) and C (0.3% trichloroacetic acid in water)	Rosmarinic acid		Antioxidant activity
90	*Phoenix dactylifera* L.)	Arecaceae [105]	Date fruits	an Atlantis C18 column (150 * 4.6 mm, 5 lm particle size)	0.1% (v/v) formic acid in water (eluent A) and acetonitrile (eluent B).	Rutin, Sinapic acid, Ferulic acid, Coumaric acid, Syringic acid, Caffeic acid, Vanillic acid, Catechin, Gallic acid		Antibacterial and cytotoxic activity
91	Kumquat (*Citrus japonica* var. margarita)	Rutaceae [106]	fruit	RP-C18 column (250 mm _ 4.6 mm, 5 lm,	A. deionized water B. acetonitrile		C-glycosides 30,50-di-C-b -glucopyrano sylphloretin (DGPP), acacetin 8-C-neohesperidoside (margaritene), acacetin 6-C-neohesperidoside (isomargaritene), apigenin 8-C-neohesperidoside, and Oglycosides, such as acacetin 7-O-neohesperidoside (fortunellin), isosakuranetin 7-O-neohesperidoside (poncirin) and apigenin 7-O-neohesperidoside (rhoifolin).	Antioxidant activity
92	*Raphanus sativus* L. var. caudatus Alef	Brassicaceae [107]		Reverse Phase-C18 column (5 lm particle size, 250 × 4.6 mm)	isocratic 5% THF-95% water	Sulforaphene Sulforaphane		Anticancer activity
93	*A. barbadensis*	Asphodelaceae [108]		An Optimapak C18column (250 × 4.6 mm, 5 µm, RStech, Seoul, Korea)	A. 0.1% phosphoric acid solution and B. 100% ace-tonitrile	3: aloin.		sorethroats and diarrhea
	Catechu	Leguminosae		As above		1: (+)-catechin, 2: (−)-epicatechin,		
	Uncaria gambir	Rubiaceae		As above		1: (+)-catechin, 2: (−)-epicatechin,		

94	Salvia cadmica	Lamiaceae [109]		Eclipse XDB C-18 reversed-phase column (250 mm × 4.6 mm length, 5 _m particle size	methanol	(1. Gallic acid, 2.Protocatechuic acid, 3. (+)-Catechin, 4. p-Hydroxybenzoic acid, 5. Chlorogenic acid, 6. Caffeic acid,7. (−)-Epicatechin, 8. Syringic acid, 9. Vanillin, 10. p-Coumaric acid, 11. Ferulic acid, 12. Sinapinic acid, 13. Benzoic acid, 14. o-Coumaric acid, 15. Rutin, 16. Hesperidin, 17.Rosmarinic acid, 18. Eriodictyol, 19. trans-Cinnamic acid, 20. Quercetin, 21. Luteolin, 22. Kaempferol, 23. Apigenin		
95	*Rheum emodi*	Polygonaceae [102]		a C18 column (250 mm x 4.6 mm; Sunfire)	A. methanol and B. 2% acetic acid	Aloe-emodin, emodin and rhein		antiviral, antimicrobial and hepato-protective activities
96	*Zanthoxylum canthopodium*	Rutaceae [110]	leaves	AcclaimTM120 C 18 column (5 µm particle size, 250 x 4.6 mm)	acetonitrile and 1% aq. the acetic acid	ascorbic acid, free phenolic acids such as gallic acid, methyl gallate, caffeic acid, syringic acid, ferulic acid, para (*p*)-coumaric acid, sinapic acid	(catechin, rutin, quercetin, myricetin, apigenin and kaempferol),	Antioxidant activity
97	*Ornithogalum species* *Ornithogalum virens*, *Ornithogalum thyrsoides, Ornithogalum dubium,*	Asparagaceae [111]	bulb	Acclaim TM 120 C18 (25 cm x 4.6 mm, 5 µm)	1% aq. acetic acid (Solvent A) and acetonitrile (Solvent B),	gallic acid, caffeic acid, p-coumaric acid, syringic acid, sinapic acid, ferulic acid,	catechin, rutin, apigenin, quercetin, myricetin, and kaempherol	useful in treatments of stomach upsets like gastric ulcers, peptic ulcers, duodenal ulcers, acidity, etc. showed anticancer, antimicrobial, cytotoxic and antioxidant properties
98	Salvia L. species namely *S. brachyantha* (Bordz.) Pobed, *S. aethiopis* L., and *S. microstegia* Boiss. and Bal.	Lamiaceae [112]	Plant powder	C18 reversed-phase Inertsil ODS-4 (150 mm _ 4.6 mm, 3 _m, GL Sciences, Tokyo, Japan) analytical column	A. water, 5 mM ammonium formate and 0.1% formic acid B. methanol, 5 mM ammonium formate, and 0.1% formic acid	apigenin, luteolin, p-coumaric acid, and chlorogenic acid.	quercetin, myricetin, and kaempherol	anticancer, antimicrobial, antioxidant properties
99	*Coffea arabica*	Rubiaceae [113]	leaves	HPLC-UV C18, reverse-phase (5 µ), Gemini column (250 × 4 mm I.D.; Phenomenex,	A. 2 % acetic acid in water B. acetonitrile	Isomangiferin . Mangiferin		health-promoting phenolic compounds.
100	*Zanthoxylum naranjillo* and *Z. tingoassuiba*	Rutaceae [114]	Leaves and stems	a Shimadzu Shim-pack CLC-Phenyl (particle diameter 5 µm, 250×4.60mm) column equipped with a pre-column and on a Phenomenex Onyx monolithic C18 (100×4.60mm) column equipped with a pre-column	A. methanol/ water (þ0.2%formic acid) B. 5 to 100% methanol	sesamin		anti-inflammatory, analgesic, and antimalarial action
101	*Equisetum arvense* L.,	Equisetaceae [115]	Strile stem	Kintex 5u RP C18 ig, 4.6 mm internal diameter × 250 mm	(A) 0.05% formic acid (HCOOH) and (B) 0.05% formic acid-acetonitrile (CH3CN),(50:50 v/v)	Synapin acid, caffeic acid, gallic acid, vanillic acid, ferulic acid, syringic acid, p-coumaric acid	Epicatechin, catechi n,quercetin,rutin,na ringenin,myricetin, luetolin	anemia, inflammation, diabetes, ulcers, cancer, convulsions, anxiety and depressive disorders
102	*Dipsacus sativus* (Linn.) Honck.	Dipsacus [116]	Dried leaves	a Waters column C18 (250 mm, 4.6 mm, 5 µm)	methanol and acetic acid in water 15:85 (v/v)	Isovitexin, Saponarin		treatment of cardio-vascular disease

103	*Salvia fruticosa* Mill.	Lamiaceae [117]	Plant powder	a reverse phase NOVA-PAK C18 column at ambient temperature (20°C).	methanol and phosphate buffer (43 : 57)		luteolin and rutin,	antioxidant and anti-inflammatory activities

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