Maria da Graça Campos
Kenneth R. Markham

Structure information from HPLC and on-line measured absorption spectra:

Flavones, Flavonols and Phenolic Acids

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E N S I N O

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"It seems to me what is called for is an exquisite balance between two conflicting needs: the most skeptical scrutiny of all hypotheses that are served up to us and at the same time a great openness to new ideas … If you are only skeptical, then no new ideas make it through to you … On the other hand, if you are open to the point of gullibility and have not an ounce of skeptical sense in you, then you cannot distinguish the useful ideas from the worthless ones."

"The Burden of Skepticism" (1987)
(CARL SAGAN).

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INTRODUCTION

This book has been compiled to provide scientists with a convenient method for the tentative identification of phenolic acids, dihydroflavonoids, flavones and flavonols in extracts of plants or plant-derived material. These compounds are virtually ubiquitous in land-based green plants (Markham, 1982) and represent about 2% of all carbon biosynthesised by plants (Smith, 1972). Representatives of all or some of these will be found in most plant extracts and are widely encountered in foods and beverages (Kyle and Duthie, 2006). Although numerous publications describing the use of absorption spectroscopy in the identification of these phenolics have appeared, (e.g. Jurd, 1962; Mabry *et al.*, 1970; Markham, 1982; Markham and Bloor, 1998), the advent of high performance liquid chromatography with on-line photo-diode array detection (HPLC/DAD) has provided a highly effective technique for the separation and tentative identification of such compounds (Markham and Bloor, 1998). This technique provides two key characters for each component, the retention time (RT) and the absorption spectrum. The combination of these two features enables the identification of the component type, and by reference to appropriate standards, provides structural information and perhaps identification. The data presented in this volume relate to a selection of authentic standard compounds from the collection of one of us (K.R.M) and will enable the reader to determine structural features of unidentified compounds through the analysis of the fine structure of the absorption spectrum. The on-line measured spectra in this book represent uniquely pure

compounds, initially purified by 2D-PC or crystallization and, for this compilation, by HPLC/DAD. Thus, the fine structure detail within spectra is reliable, and many correlations based on this are described here for the first time. Previous compilations of spectra, typically derived from samples purified by 2D-PC or crystallization, are less reliable in this respect. Additionally, such spectra are measured in solvents which differ from those used for HPLC. This can affect peak maxima and fine structure detail.

CHAPTER 1

1. Origin and function of flavonoids and phenolics acids.

Representatives of the groups of natural products chosen for this compilation are found in all land-based green plants. They are all products of the shikimate (phenylpropanoid) biosynthetic pathway (Haslam, 1974; Dewick, 2001), which produces cinnamic acid (via phenylalanine). Cinnamic acid gives rise to both benzoic acids and hydroxycinnamic acids. One of the latter, 4-hydroxycinnamic acid, as its coenzyme A derivative, is the product which, when elaborated further by interaction with three malonyl-coenzyme-A units, produces the primary flavonoid (chalcone/flavanone) from which all other flavonoids are derived (Heller and Forkman, 1994; Davies and Schwinn, 2006). The most common of these flavonoids by far, are the flavones and flavonols which occur almost exclusively as glycosides within plants and as aglycones on the plant surface. Flavonoids other than those studied here, e.g. isoflavonoids, anthocyanins, aurones etc., have a more restricted distribution in the plant kingdom and are frequently associated with specialized plant organs.

Flavonoids (and to some extent phenolic acids), perform a range of functions in green plants (Gould and Lister, 2006). Protective functions include UV screening, free radical scavenging and anti-microbial and anti-feedant activity. Signalling functions include flower and fruit colouration, involvement in the initiation of root nodule formation and pollen tube growth. They also function as controllers of enzyme activity. Some of

these activities have found application in the promotion of human health and well-being (Clifford and Brown, 2006). Their free radical scavenging capability in particular, is one of the major reasons why foods rich in flavonoids such as fruit, vegetables, tea, red wine, etc., are thought to protect against (oxidative) degenerative diseases such as arthritis, cancer and hypertension. Vitamin sparing and regeneration is thought to be another function of flavonoids in the diet.

2. Use of this book

Providing that the HPLC methodology used here for obtaining RTs and absorption spectra is adhered to, the data obtained in an analysis may be directly compared with the data presented here. The first step in a tentative identification involves interpretation of the absorption spectrum to determine compound type, i.e. aromatic acid, dihydroflavonoid, flavone, flavonol, or "other". Comparison with reference spectra in this compilation should provide this information. To aid in compound identification two tables of reference compounds are included, one (Table 1) in which the reference compounds are grouped according to general compound type, e.g. aromatic acids, dihydroflavonoids, flavones, and flavonols, and another (Table II) in which they are listed in order of RT. The absorption spectra are also reproduced so that fine structural features of spectra can be seen. Common names are used where available and structures represented by such names may be found in chemical supply catalogues (aromatic acids) or standard flavonoid texts, (e.g. Hattoti, 1962; Grippenberg, 1962; Markham, 1982; Harborne, 1994, Campos, 2005).

When structure information on an unknown compound in one of the above categories is required, the first step using the information in this compilation would be to compare the absorption spectrum (of the glycoside or its derived aglycone) with those reference spectra presented here. If an exact match can be found, then a comparison of RTs will give an

indication of whether the unknown is likely to be identical to the reference. If both the absorption spectrum and the RT match, then proof of structure would require co-chromatography of the unknown with an authentic sample (a range of commercial sources of such compounds is provided herein). Co-injection of equivalent amounts of both compounds should produce a single, clean peak if they are identical.

If an exact match for the spectrum can be found, but the RT differs, then it is likely that the unknown differs from the reference only in the number or nature of sugars attached to the glycosylated site, or in the nature of a substituent, *e.g.* *O*-methyl compared with *O*-ethyl, or presence/absence of an *aliphatic* acyl group on a sugar. If the unknown is eluted faster than the reference (*i.e.* has a lower RT), then because the chromatographic solid phase is non-polar relative to the polar solvent, this indicates that the unknown is more polar (*e.g.* contains more sugars or a sugar of higher polarity). Conversely if the unknown is slower eluting than the reference (*i.e.* higher RT), then the unknown is less polar (e.g. contains fewer sugars or a sugar of lower polarity or contains an aliphatic acyl group).

In the event that no exact match for the absorption spectrum can be found amongst the reference spectra, a close match may indicate that the unknown is related to the reference, perhaps with the same oxygenation pattern but with a different substitution pattern. In the accompanying text, a number of rules are outlined which will help define the effects expected from varying substitution patterns.

With regard to compound identification, a word of caution. As has been stated previously (Markham, 1982), reliable proof of identity of an unknown with a reference compound requires comparison via a minimum of three characters. RT and an absorption spectrum comprise only two, and ideally any identification should include one other technique as well. For example TLC, 2D-PC, a mass spectrum or an nuclear magnetic resonance spectrum, or hydrolysis and product identification (by HPLC).

For further information on the application of these techniques to flavonoid structure elucidation see Markham and Geiger, 1994 (^{1}H-nmr), Agrawal, 1989, and Markham and Chari, 1982 (^{13}C-nmr), Mabry and Markham, 1975, and Wolfender *et al.*, 1992 (mass spectrometry) and Markham, 1982 (chromatography and hydrolysis).

3. Chromatographic methodology

Retention times for the reference standards in this compilation were measured on a Merck Lichrospher 100 end-capped RP-18 column (11.9 x 0.4 cm, i.d. 5μm) using a Waters (Milford, MA, USA) 600E solvent controller, a Waters 996 photodiode array detector, a Jasco (Tokyo, Japan) 851-AS intelligent sampler and a Millennium^{32} (Milford, MA, USA) Version 3.05.01 software. Elution was carried out using a flow rate of 0.8 ml.min^{-1} at 24°C.

The solvents comprised water adjusted to pH 2.5 with orthophosphoric acid (A) and acetonitrile (B) mixed using a linear gradient starting with 100% A, decreasing to 91% over the next 12 min to 87% over the next 8 min and to 67% over the next 10 min. After holding the solvent at this composition for 2 min, A was decreased to 57% over the next 10 min, and then held at this level until the end of the 60 min analysis.

Spectral data for all peaks were accumulated in the range 220-400 nm. The linearity of the detector response was checked with the standards, quercetin, quercetin-3-*O*-rhamnoside (quercetrin) and quercetin-3-*O*-rutinoside (rutin) (Amiot *et al.*, 1988; Campos *et al.*, 1990; Campos, 1997).

Note that if an RP-18 column with different dimensions is used, the retention time data obtained will be either higher (columns of larger size) or lower (columns of smaller size), but the sequence of retention times of the same mixture of compounds will remain the same.

4. General notes on spectra and presentation

Throughout this book reference is made to the sites of substitution in flavonoids, to the A-, B- and C- rings of flavonoids, and to bands I, IIa and IIb in the absorption spectrum and hypsochromic and bathochromic shifts in these bands. Substitution sites are defined by the number of the carbon atom, and the numbering of carbon atoms on the flavonoid nucleus is illustrated in figure 1.

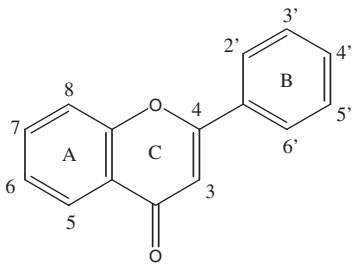

Figure 1: Flavone nucleus

Also in this figure are the designations of the A-, B- and C- rings. Figure 2 illustrates certain features of the flavonoid absorption spectrum, including the band I, IIa and IIb definitions, a commonly encountered shoulder, and an instrument generated aberration. This aberration is visible in a number of spectra at about 370nm and is caused by the changeover from the UV to the visible lamp in the detector. This may take the form of a shoulder on the side of a peak or a sharp valley near the apex of a peak. Other detectors may or may not produce this, but it has been retained in the spectra presented here so as to ensure no loss of spectral accuracy.

When comparisons of two or more spectra are discussed in the text, the difference between the wavelength of the same absorption peak in each spectrum is referred to as a *hypsochromic* or *bathochromic* shift. The former refers to a shift to a lower wavelength and the latter to a shift to higher wavelength.

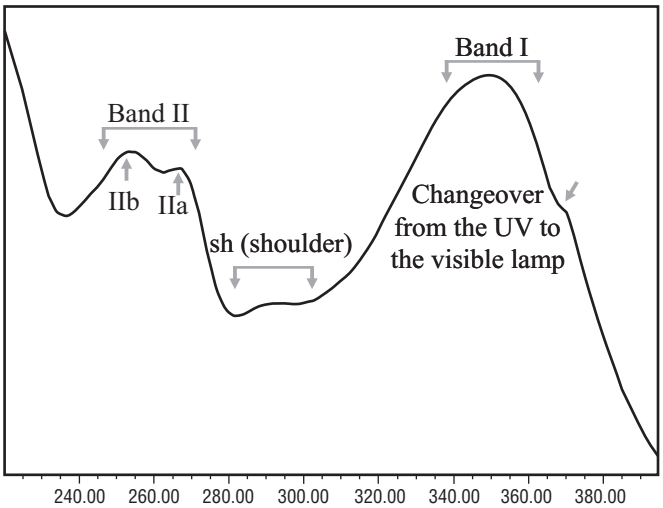

Figure 2 – Illustration of certain features of the flavonoid absorption spectrum, including the band I, IIa and IIb designations, shoulder examples including the changeover from the UV to the visible lamp.

5. Useful sources of reference compounds (not exhaustive)

Apin Chemicals Ltd (UK): http:// www.**apinchemicals**.com

Carl Roth GmbH + Co.: http://www.**carl-roth**.de

INDOFINE Chemical Company, Inc.: www.**indofinechemical**.com

Merck: http:// www.**merck**.com
e-mail: service@merck.de
telefax: 0049-6151 722000

Plantech(UK): www.**plantech**ltd.co.**uk**
e-mail: plantechUK@aol.com

Extrasynthese (France): www.**extrasynthese**.com

Sigma: www.**sigma-aldrich**.com/order

CHAPTER 2

1. PHENOLIC ACIDS

Phenolic acids of the benzoic or cinnamic acid type are commonly found as derivatives in plant extracts and are frequently encountered as acyl groups attached to flavonoid glycosides. For analysis these are readily cleaved from the glycoside with alkali (see Markham, 1982). In some respects their absorption characteristics are analogous to the B-ring contribution in the spectra of flavones and flavonols. When the substitution pattern is symmetrical in benzoic acids and in some cinnamic acids, for example in benzoic (**1**), gallic (7), cinnamic (**9**), *p*-hydroxybenzoic (**3**), syringic (**8**) and sinapic (**16**) acids, the spectrum is comprised of only one absorption band.

In the simplest example, benzoic acid, the spectrum displays a single absorption band at 272nm. This spectrum is not noticeably altered by the introduction of the conjugated side-chain in cinnamic acid, and in fact the easiest way to distinguish these acids by HPLC/DAD is from their very different retention times of 31.5 min. (benzoic) and 41 min. (cinnamic).

Hydroxylation of benzoic acid at the *para* position produces a 19nm hypsochromic shift of the major absorption band from 272nm to 255nm whereas in cinnamic acid, *para*-hydroxylation produces the reverse effect, a bathochromic shift of similar proportions (**cf. spectra 1, 3 and 9, 12**). When hydroxylation/methoxylation substitution is unsymmetrical, e.g. 2,4-dihydroxybenzoic acid (4), a double absorption peak may be produced. This phenomenon is also seen in the spectra of flavones and flavonols (q.v.).

The monohydroxylated cinnamic acids, *ortho*-, *meta*- and *para*-coumaric acids (**10, 11** and **12**) are readily distinguished by their absorption spectra. In general terms, as the distance between the hydroxyl and the side chain decreases (i.e., from *para* to *meta* to *ortho*), the separation of the two absorption bands increases. The commonly encountered *para*-coumaric acid possesses a characteristic double peak absorption at about 293sh and 308nm which readily identifies the presence of it or its derivatives in acylated flavonoids and anthocyanins. The spectra of the *ortho*- and *meta*- coumaric acids retain a reduced absorption at about 320nm and exhibit their other major absorption band at significantly lower wavelengths than *para*-coumaric acid.

Caffeic acid is often found as its 3-methyl ether, ferulic acid (**14**) in acylated flavonoids. The spectrum of ferulic acid is very similar to that of caffeic, but ferulic acid can be readily distinguished by its longer retention time (31,4 min. versus 23,5 min.). Caffeic acid 4-methyl ether, isoferulic acid (**15**) is also difficult to distinguish by its absorption spectrum alone. However, isoferulic acid is rarely, if ever, encountered as an acyl group in flavonoids. It can only be distinguished from ferulic acid with any certainty by co-HPLC with an authentic standard. Flavonoid glycosides acylated with ferulic acid are readily distinguished from the equivalent caffeoylated flavonoid glycosides, by their significantly higher retention times.

2. DIHYDROFLAVONOIDS

The dihydroflavonoids possess spectra rather similar to those of the benzoic acids. This is due to the fact that the major chromophore in dihydroflavonoids is the A-ring which is isolated electronically from the lower absorbing B-ring. The spectrum of a 5-deoxy-dihydroflavone like fustin (**22**) could be confused with that of *ortho*-coumaric acid (**10**), however these compounds are readily distinguished by their very different

retention times. Also similar are the spectra of naringenin (**19**), eriodictyol (**20**), and dihydroquercetin (**23**) and *meta*-coumaric acid. Again, these are generally easy to distinguish from their retention times, but dihydroquercetin and *meta*-coumaric acid have similar RTs and are difficult to distinguish.

7-*O*-glycosylation of dihydroflavonoids (e.g. as in eriodictyol-7-*O*-neohesperidoside, **21**) has little effect on the absorption spectrum. Nor does the conversion of a dihydroflavone to a dihydroflavonol (e.g. eriodictyol, **20**, to dihydroquercetin, **23**) through the addition of a hydroxyl at C-3. HPLC retention times however do permit distinctions, e.g. eriodictyol (RT = 40,8 min.) and dihydroquercetin (RT = 32,8 min.). Interestingly eriodictyol-7-*O*-neohesperidoside (**21**) has the same retention time (and spectrum) as dihydroquercetin and these would need to be distinguished via acid hydrolysis to produce the HPLC distinguishable aglycones.

3. FLAVONES

The absorption spectra of flavones and flavonols typically exhibit two bands, band I in the range 320-385nm and band II in the range 250-285nm. When flavones lack B-ring substitution, or possess a 4'-oxygenated B-ring, band II of the spectrum appears as only one peak. The introduction of additional B-ring oxygenation commonly produces a double band II (bands IIa and IIb). Band IIa may appear simply as a shoulder on the long wavelength side of band IIb (see later discussion).

3.1. Flavones with minimal or unusual oxygenation

When oxygenation is present only in the A-ring as in chrysin (**25**) and baicalein (**26**) and includes a 5-hydroxyl, the spectrum is dominated by band II. The introduction of B-ring oxygenation as in 5-hydroxy-4'-

methoxyflavone (**24**) however, enhances the intensity of band I. In the spectrum of 5,7,2'-trihydroxyflavone (**27**), relative to that of chrysin (**25**), the introduction of the 2'-hydroxyl has produced a marked enhancement of band I, with little change being evident in band II. This enhancement in band I is even greater when the B-ring oxygenation is at C-4' as in apigenin (**28**).

In baicalein (**26**), which lacks B-ring oxygenation but contains an additional A-ring hydroxyl relative to chrysin, band II exhibits a significant bathochromic shift (277nm in baicalein, compared with 266nm in chrysin).

3.2. The apigenin (5,7,4') oxygenation pattern

The absorption spectra of flavones with the 5,7,4' oxygenation pattern are characterized by two major absorption bands, band I at 325-337nm and band II at 265-275nm (see apigenin, **28**). Derivatisation of one or more of the three hydroxyls of apigenin can be detected from slight changes in this spectrum. For example 7-*O*-glucosylation (see apigenin-7-*O*-glucoside, **31**, and apigenin-7-*O*-apiosylglucoside, **30**) causes the 290nm shoulder visible in the spectrum of apigenin, **28**, to disappear. 7-*O*-methylation however does not produce this effect (see genkwanin, **36**). Methylation at the 4'-hydroxyl is evident from a marked hypsochromic shift in band I of 7-8nm (compare acacetin-7-*O*-neohesperidoside, **37**, and apigenin-7-*O*-neohesperidoside, **29**).

3.2.1. *6- and 8-substitution effects*

6- and 8-*C*-glycosylation has little or no effect on band I absorption, and the rules defined earlier for the effect of a second B-ring hydroxyl on band still apply (see spectra of vitexin, **32**, and orientin, **52**).

The effect of 6-*C*-glycosylation on band II however differs from that of 8-*C*-glycosylation. 6-*C*-glycosylation (e.g. in isovitexin, **33**, and isorientin, **53**) causes an apparent bathochromic shift in the major peak in band II

relative to the aglycone, where as 8-*C*-glycosylation (e.g. in vitexin, **32**, and orientin, **52**) has little if any effect.

Further *O*-glycosylation of the sugar in vitexin (see 2''-*O*-glucosylvitexin, **34**) also has no effect on the absorption spectrum. Indeed the spectra of apigenin, vitexin and 2''-*O*-glucosylvitexin are indistinguishable. These compounds however are readily distinguished from their retention times of 45.1, 32.6 and 31.7 min. respectively.

Methoxylation at C-6 produces a bathochromic shift in band II similar to that seen with 6-*C*-glycosylation (see scutellarein – 6 , 4'-dimethylether, **40**).

Two examples of 8-oxygenated apigenins are presented here, **38** and **39**. The spectrum of 8-hydroxyapigenin-8-*O*-glucuronide (**38**) resembles that of the apigenin itself, and it appears that 8-*O*-glycosylation of 8-*O*-hydroxyapigenin negates the effect of 8-hydroxylation on the aglycone, apigenin (**28**). In contrast, the spectrum of a 7-*O*-glycosylated 8-hydroxyapigenin such as 8-hydroxyapigenin-7-O-allosylglucoside (**39**) reflects the effect of 8-hydroxylation. This spectrum possesses a most unusual set of absorption peaks with a major (additional) peak at ca. 300nm. Similar distinctions between these two different glycosylation patterns and their effects on absorption spectra have been observed previously (Markham and Given, 1988).

3.3. The luteolin (5,7,3',4') oxygenation pattern

The absorption spectra of flavones with the 5,7,3',4'-oxygenation pattern typically possess three absorption peaks at about 254, 267 and 345-350nm (see luteolin, **42**) The doubled band II, bands IIa and IIb, seem to be characteristic of the 3',4'-dioxygenation pattern compared to the 4'-oxygenation pattern in apigenin. As in the spectrum of apigenin, a shoulder at around 290nm is evident in the spectrum of luteolin. 7-*O*-Glycosylation of luteolin (see luteolin-7-*O*-glucoside, **43**), luteolin-7-*O*-

rutinoside, **46**, and luteolin-7-*O*-glucurono-glucuronide, **47**) causes this shoulder to disappear as was seen with apigenin. When glycosylation is present at both the 7- and the 4'-hydroxyls, the spectrum is modified such as to appear like that of a 7-*O*-glycosylated tricin (see luteolin-7,4'-di-*O*-glucuronide, **48**, and tricin-7-*O*-rhamnoglucuronide, **71**). The retention times however readily distinguish these compounds (30.7 and 36.2 min. respectively).

3'-*O*-Glycosylation has a dramatic effect on the spectrum of luteolin. Band IIa at 268nm appears very prominent through a hypsochromic shift of band IIb to around 240nm. Band I also suffers a hypsochromic shift to 342nm (see luteolin-3'-*O*-glucoside, **44**). The spectrum in effect becomes more apigenin-like with the 3'-*O*-glycosylation negating the influence of the 3'-hydroxyl on the spectrum. Additional 7-*O*-glycosylation (see luteolin-7,3'-*O*-diglucuronide, **49**) causes the disappearance of the *ca.* 290nm shoulder as has been observed before with 7-*O*-glycosylation.

As with 3'-*O*-glycosylation, 4'-*O*-glycosylation of the luteolin nucleus produces an enhancement of band IIa (e.g. in luteolin-7,4'-di-*O*-glucuronide, **48**), but unlike 3'-*O*-glycosylation, glycosylation at the 4'-hydroxyl produces a sizeable hypsochromic shift in band I. When both the 3'- and 4'-hydroxyls are glycosylated as in luteolin-3',4'-di-*O*-glucuronide (**50**), the enhancement of band IIa is accompanied by a marked drop in the intensity of band I as well as a major hypsochromic shift. The overall effect is to reduce the influence of the 3'- and 4'-hydroxyl groups on the spectrum such that in general form it approaches that of 5,7-dihydroxyflavone (chrysin, **25**). Additional 7-*O*-glycosylation as in spectrum **51**, has little further discernable effect.

5-*O*-glycosylation of luteolin produces a spectrum similar to that of luteolin and, because luteolin-5-*O*-glucoside (**45**) lacks 7-*O*-glycosylation, the spectrum also contains a shoulder at *ca.* 290nm. A distinctive shoulder at 240nm appears in the spectrum of luteolin-5-*O*-glucoside (and in chrysoeriol-5-*O*-glucoside, **56**).

O-Methylation of luteolin at the 3'-hydroxyl (see chrysoeriol) has little effect on the absorption spectrum apart from deepening the valley between bands IIa and IIb at 269 and 252nm respectively. This relative lack of effect is evident also from a comparison of the 5-*O*-glucosides of luteolin and chrysoeriol (**45** and **56**), and the 6,8-di-*C*-glucosides of both (**54** and **58**).

3.3.1. *6- and 8-substitution effects*

For 6- and 8-*C*-glycosylation effects refer to section 3.2.1 above.

Additional 6- or 8-oxygenation produces its main effects on band II (see 6-hydroxyluteolin, **61**). In the spectrum of 6-hydroxyluteolin (**61**), band IIa is shifted to about 278nm from near 267nm in luteolin (**42**). Glycosylation at the 7-hydroxyl is evidenced, as in the apigenins and luteolins, by a loss of the slight shoulder at about 310nm and by a marked enhancement of band IIa (see the 7-glucosylglucoside, **62**, 7-xylosylglucoside, **63**, and 7-xylosylxyloside, **64**, of 6-hydroxyluteolin). These compounds although not readily distinguishable from their spectra, possess significantly different RTs of 29.7, 38.7, and 34.9 min. respectively.

The only 8-oxygenated luteolin aglycone derivative included in this work is 7-hydroxy-5, 8, 3', 4'-tetramethoxyflavone (**74**), but because of its extensive methylation, spectral shifts relative to luteolin have little significance. Comparison with the only other 5-*O*-substituted luteolin, the 5-*O*-glucoside, (**45**), suggest that the major effect of 8-oxygenation is, as with 6-oxygenation, a bathochromic shift and enhancement of band IIa. This effect is also seen in 8-hydroxyluteolin when glycosylated at the 7-hydroxyl (**65**), but not when glycosylated at the 8-hydroxyl (**66**). In fact when 8-hydroxyluteolin is glycosylated at the 8-hydroxyl, the spectrum strongly resembles that of luteolin (**42**) as was the case in the analogous apigenin series (q.v.). Thus, such glycosylation appears to negate the effect of 8-hydroxylation on the luteolin spectrum which is so evident with the 7-*O*-glucoside (**65**). Data supporting this conclusion have been published by Markham and Given (1988).

3.4. The tricetin (5, 7, 3', 4', 5') oxygenation pattern

The spectrum of tricetin (**67**) differs from that of the related luteolin (**42**) in having its major band IIa absorption at 266nm and in the relative increase in the intensity of band I at 350nm. The shoulder at ca. 290nm diminishes in size on glycosylation at the 7-hydroxyl (see tricetin-7-*O*-glucoside, **68**), as has been observed in the apigenin and luteolin series. Comparison of the spectra of tricetin (**67**) and its 3',5'-dimethyl ether tricin (**69**), reveals that these two compounds (and their equivalent derivatives) are not easy to distinguish from their absorption spectra alone, although bands IIa and IIb do appear better defined in the latter. However they are well separated on HPLC with retention times of 38 and 46 min respectively. Glycosylation of tricin at the 5-hydroxyl (see tricin-5-*O*-glucoside, **70**) has little effect on the absorption spectrum while glycosylation at the 7-hydroxyl brings about the same change as observed previously, i.e. the diminution of the shoulder at ca. 296nm. Glucosylation at the 5-hydroxyl has a major effect on the polarity, judging by the large change in retention time of about eleven minutes, an effect comparable with that of 7-*O*-glycosylation (cf. tricin-7-*O*-rhamnosylglucuronide, **71**).

When tricetin is *O*-methylated at the 3', 4', and 5'-hydroxyls a major change in the spectrum is observed. A hypsochromic shift in band I of 19nm relative to tricetin, and 23nm relative to tricin is produced, together with a marked decrease/loss of band IIb (see spectrum **72**). The spectral change is remarkable, and reduces the absorption spectrum to one resembling genkwanin (**36**), a flavone whose retention time of 57.1 min. is not too dissimilar from that of tricetin-3',4',5',-trimethylether at 54.7 min.

3.4.1. *6- and 8- oxygenation effects*

In the only example in this compilation, scaposin (**73**), the major effect of introducing methoxyl groups at C-6 and C-8 appears to be a bathochromic shift of band II to around 280nm. The hypsochromic shift

of band I to about 330nm is probably caused by methylation of the 4'-hydroxyl group as seen in the spectrum of tricetin-3',4',5'-trimethyl ether (**72**). It is noteworthy that even in the absence of 7-*O*-glycosylation, no shoulder is evident in the 295nm region. Additional relevant information regarding the effects of introducing oxygenation at C-6 and C-8 is presented for the flavonols (Section 4.1).

4. FLAVONOLS

4.1. Simple unusual oxygenation patterns

When 3-hydroxyflavone [$\lambda_{max.}$ 239, 243sh, 306, 344nm (Mabry *et al.*, 1970)] is substituted with a hydroxyl group at C-2', the main effect on the spectrum is a decrease in the intensity of band I relative to band II. In 2'-hydroxyflavonol (**75**), the same three absorption bands as in 3-hydroxyflavone are present, but at somewhat shorter wavelengths.

Oxygenation of 3-hydroxyflavone at the 3' and 4' positions in contrast, increases the intensity of band I such that the intensity exceeds that of band II (see 3',4'-dihydroxyflavonol, 77).

Of interest in the spectrum of 3',4'-dihydroxyflavonol (77), is the lack of a doubled band II which is seen in most other 3',4'-dioxygenated flavonoids. It would seem that the presence of a 5-hydroxyl is a necessary prerequisite for this doubling to appear, at least in 3-hydroxyflavones (see also fisetin, **82**, and 3',4'-dimethoxyflavonol **78**). The absence of a 5-hydroxyl also appears to enhance the shoulder between bands I and II, i.e. in the 290-315 range (compare fisetin, **82** with quercetin, **108**, and 7,8,4'-trihydroxyflavonol, **80**, with the herbacetin derivatives, **103** and **104**).

The absence of B-ring oxygenation as in galangin, **76**, surprisingly has little effect on the form of the spectrum (cf. kaempferol, **83**) although the relative intensity of band I is significantly reduced.

4.2. The kaempferol (3,5,7,4') oxygenation pattern.

The spectrum of kaempferol (**83**) resembles that of 5,7-dihydroxyflavonol (galangin, **76**), but has a more intense band I. This spectrum is largely unaffected by 4'-*O*-methylation (see 4'-*O*-methylkaempferol, **97**), but undergoes a noticeable change when kaempferol is derivatized at the 3-hydroxyl. With 3-glycosylation band I undergoes a hypsochromic shift of some 15-20nm, and this is accompanied by a relative drop in intensity (see kaempferol glycosides **84, 85, 87, 88** and **89**). When rhamnose is the sugar, this hypsochromic shift is somewhat greater (see comment on quercetin in section 4.3). Distinction of kaempferol-3-*O*-rutinoside (**89**) from the 3-*O*-neohesperidoside, **88** (which possess the same absorption spectra and produce the same hydrolysis products) is easily achieved from the retention time. The 1-6 linkage (rutinose) causes the molecule to elute about 2 min. later than the 1-2 (neohesperidose) linkage.

7-*O*-glycosylation of kaempferol as in kaempferol-7-*O*-glucoside (**86**) has very little effect on the absorption spectrum, and thus it is not surprising that when kaempferol is glycosylated at both the 3- and 7-hydroxyls, (spectra **92, 93,** and **94**), the spectra resemble those of kaempferol 3-*O*-glycosides. An exception is the sinapoylated derivative, (**96**), where the superimposition of the spectrum of the sinapoyl group produces both an enhanced band I and caused an apparent shift back to 331nm. The nature or existence of any acylation however may not be evident from the absorption spectrum, e.g. in kaempferol-3-*O*-(6-acetylglucoside)-7-*O*-glucoside (**94**). In such cases, RTs values are often of value since acylation normally increases the retention time, with each acyl group increasing it to a different extent.

4.2.1. *8-substitution-effects*

8-hydroxylation of kaempferol produces herbacetin. Its 7-*O*-methyl ether (**105**) has a spectrum quite different from that of kaempferol. In

particular, both bands I and II exhibit a bathochromic shift and the shoulder(s) in the 320nm region are markedly enhanced. Methylation of the 8-hydroxyl makes little difference in the absorption spectrum, but the retention times permit ready distinction (see 8-*O*-methyherbacetin, **103**). The spectrum is also barely changed by 8-*O*-glucosylation, but here again the retention time permits ready distinction (see 8-*O*-glucosylherbacetin, **101**). Additional 3-*O*-glycosylation in an 8-*O*-substituted herbacetin however brings about a notable change in the absorption spectrum. In both herbacetin-3-*O*-glucoside-8-*O*-glucuronide (**102**) and 8-*O*-methylherbacetin-3-*O*-sophoroside (**104**), band I has suffered a major drop in intensity and a 15-20nm bathochromic shift, changes also observed with some other 3-O-glycosides.

3-*O*-Glycosylation of 7-*O*-methylherbacetin (**105**) (itself producing an unusual spectrum), produces a very unusual, almost non-flavonoid absorption spectrum (**106**). In the only example of 7,8,3-substitution of herbacetin, 7,8-di-*O*-methylherbacetin-3-*O*-(2-*O*-ferulylglucoside), (**107**), the spectrum has reverted to what appears to be an apigenin type. However, with band(s) I in the 7- and 7,3-disubstituted herbacetins being of relatively low intensity, it seems likely that in **107** these bands have been masked by the absorption of the ferulic acid acyl group which would absorb strongly at 329nm.

4.3. The quercetin (3,5,7,3',4') oxygenation pattern

With 3',4'-dihydroxylation in the B-ring, quercetin (**108**) exhibits a doubled band II, although band IIa appears only as a shoulder on the dominant band IIb. It should be mentioned that occasionally kaempferol glycosides also exhibit bands IIa and IIb (see kaempferol-7-*O*-glucoside, **86**), although a single band II absorption is the norm for kaempferol glycosides. As with kaempferol, 3-*O*-glycosylation of quercetin causes a hypsochromic shift in band I together with a reduction in intensity rela-

tive to band II (see quercetin-3-*O*-galactoside and quercetin-3-*O*-glucoside, **109**, **110**,). This is even more marked in the 3-*O*-rhamnoside (**112**) spectrum in which the hypsochromic shift is *ca*. 25nm compared with *ca*. 18nm with the other 3-*O*-glycosides. It is thought that the α-linkage of the rhamnose to the 3-hydroxyl causes the sugar to interfere with the free rotation of the B-ring in this compound. This in turn leads to a reduction in π-electron overlap between the B- and C-rings.

In the series of 3-*O*-glycosides studied, although most of the absorption spectra are indistinguishable, the sugar moiety can be determined from the HPLC RTs. Thus the galactoside = glucoside < arabinoside < rhamnoside, a sequence probably true also for glycosides of other flavonoids.

7-*O*-Glycosylation has little effect on the absorption spectrum of quercetin (see quercetin-7-*O*-glucoside, **113**) apart from the disappearance of the shoulder at about 295nm (as observed with the flavones).

Permethylation of quercetin (see quercetin-pentamethylether, **124**) produces the expected hypsochromic shifts in both bands.

The effects of methylation of the 3'- and 4'-hydroxyl groups in quercetin are evident from a comparison of the equivalent quercetin (**108**), isorhamnetin (3'-*O*-Me) (**118**) and tamarixetin (4'-*O*-Me) (**122**) spectra. Thus, when the spectra of the isorhamnetin 3-*O*-galactoside (**119**), 3-*O*-glucoside (**120**) and 3-*O*-rutinoside (**121**) are compared with those of 3-glycosylated quercetins (e.g. **109**, **110**), the differences are slight. One minor difference is a 3nm hypsochromic shift in the band I absorption of the isorhamnetins, and another is the resolution of bands IIa and IIb, which is more pronounced in the isorhamnetin glycosides.

Methylation at the 4'-hydroxyl however, produces no obvious change in the quercetin spectrum (see the spectra of quercetin-7-*O*-glucoside, **113**, and tamarixetin-7-*O*-rutinoside, **122**). *O*-Methylation does normally cause an increase in the retention time (e.g. quercetin, 41.9 min; isorhamnetin, 47.3 min; 3-*O*-methylquercetin, 43.4 min.) and so, the similar absorption spectra should not lead to miss-identification.

Further methylation of isorhamnetin glycosides at the 5-hydroxyl (e.g. in 5-*O*-methylisorhamnetin-3-*O*-glucoside **123**) does not noticeably affect the form of the isorhamnetin spectrum (see isorhamnetin-3-*O*-glucoside, **120**). The effect of 5-*O*-methylation on the RT at around 2.5 min. is comparable to that seen for methylation at other hydroxyls in quercetin glycosides.

4.3.1. *6- and 8-substitution-effects*

The major effect of the introduction of 6-oxygenation into quercetin can be seen in the comparison of quercetin-7-*O*-glucoside (**113**) and quercetagetin-7-*O*-glucoside (**127**). Whilst a hypsochromic shift is evident in band I, band II has undergone a noticeable bathochromic shift and band IIa has intensified. These effects are not seen with the introduction of a 6-methoxyl however. Thus the spectrum of patuletin-7-*O*-glucoside (**126**) is essentially indistinguishable from that of quercetin-7-*O*-glucoside (**113**).

8-Hydroxylation of quercetin, as in gossypetin-7-*O*-glucoside (**128**), produces a much more marked effect than 6-hydroxylation. In comparison with quercetin-7-*O*-glucoside (**113**), spectrum **128** displays a bathochromic shift in band II with a major increase in the intensity of band IIa as seen with 6-hydroxylation (spectrum **127**). 8-Hydroxylation also causes an 11nm bathochromic shift of band I together with a major intensification of a band at 340nm which is not seen in the quercetin equivalent. When gossypetin is glycosylated at the 8-hydroxyl (e.g. in the 8-*O*-glucoside-3-*O*-sophoroside, **129**), the spectrum reverts to that of a quercetin-3-*O*-glycoside type, almost as if the influence of the 8-hydroxyl had been largely negated by 8-glycosylation.

4.4. The myricetin (3,5,7,3',4',5') oxygenation pattern

A comparison of the spectra of quercetin (**108**) and myricetin (**132**) reveals that the extra oxygenation at C-5' in the latter has little effect on

the spectrum apart from perhaps a slight enhancement of the band IIa shoulder. This is true also for the equivalent glycosidic forms (cf. myricetin-3-*O*-galactoside, **133**, and 3-*O*-rhamnoside, **134**, with the quercetin equivalents, **109** and **112**).

REFERENCES

Agrawal, P.K. (1989), Editor, Carbon-13 nmr of flavonoids. Elsevier, Netherlands.

Amiot *et al.,* (1988) – Importance of flavonoids in sunflower honeys, *Bull. de Liaison du Groupe Polyphenols*. 14, 330-331.

Campos M., *et al.,* (1990) – Caracterization of flavonoids in three hive products : Bee pollen, Propolis, and Honey. *Planta Medica*, 56, 580-581.

Campos M., (1997). Caracterização do polen apicola pelo seu perfil em compostos fenolicos e pesquisa de algumas actividades biologicas. PhD- thesis. Faculty of Pharmacy – University of Coimbra, Portugal 97-99; 111-126.

Campos M., (2005). Flavonoids. In "Farmacognosia e Fitoquimica". (Proença da Cunha A, eds) Fundação Calouste Gulbenkian; 237-289.

Clifford, M.N. and Brown, J.E. (2006). Dietary flavonoids and health – broadening the perspective. In,"Flavonoids – Chemistry, Biochemistry and Applications" (O.M.Andersen and K.R.Markham, eds), CRC Press, Taylor and Francis Group, Boca Raton,FL, 319-370.

Davies, K.M. and Schwinn,K.E. (2006). Molecular biology and biotechnology in flavonoid biosynthesis. In," Flavonoids – Chemistry, Biochemistry and Applications" (O.M.Andersen and K.R.Markham, eds), CRC Press, Taylor and Francis Group, Boca Raton,FL, 143-218.

Dewick, P. M. (2001). Medicinal natural products: a biosynthetic approach. 2nd ed. – Chichester: John Wiley.

Gould, K.S. and Lister, C. (2006). Flavonoid functions in plants. In," Flavonoids – Chemistry, Biochemistry and Applications" (O.M.Andersen and K.R.Markham, eds), CRC Press, Taylor and Francis Group, Boca Raton,FL, 397-442.

Grippenberg, J.(1962). Flavones. In,"The Chemistry of Flavonoid Compounds" (T.A.Geissman, ed). Pergamon Press, N.Y. 406-440.

Harborne, J.B. (1994). Editor, The Flavonoids – Advances in Research since 1986, Chapman and Hall, London. Chapters therein.

Haslam, E (1974). "The Shikimate Pathway", Butterworth and Co. London

Hattori, S. (1962). Glycosides of flavones and flavonols. In,"The Chemistry of Flavonoid Compounds" (T.A.Geissman, ed).Pergamon Press, N.Y. 317-352.

Heller, W. and Forkman, G. (1994). Biosynthesis of flavonoids. In, The flavonoids – Advances in research since 1986 (J.B. Harborne, ed), Chapman and Hall, London, 499-531.

Jurd, L. (1962). Spectral properties of flavonoid compounds. In, The chemistry of flavonoid compounds (T.A. Geissman, ed.), Pergamon Press, London, 107-155.

Kyle, J.A.M. and Duthie, G.G. (2006). Flavonoids in foods. In," Flavonoids – Chemistry, Biochemistry and Applications" (O.M.Andersen and K.R.Markham, eds), CRC Press, Taylor and Francis Group, Boca Raton,FL, 219-262.

Mabry, T.J., Markham, K.R. and Thomas, M.B. (1970). The systematic identification of flavonoids, Springer Verlag, New York.

Mabry, T.J. and Markham, K.R. (1975). Mass spectometry of flavonoids. In, The flavonoids (J.B. Harbone, T.J. Mabry and H. Mabry, eds), Chapman and Hall, London, 78-126.

Markham, K.R. (1982). Techniques of flavonoid identification, Academic Press, London.

Markham, K.R. and Bloor, S.J. (1998). Analysis and identification of flavonoids in practice. In, Flavonoids in health and disease (C.A. Rice-Evans and L. Packer, eds), Marcel Dekker Inc., New York, 1-33.

Markham, K.R. and Chari, V.M. (1982). Carbon-13 nmr spectroscopy of flavonoids. In, The Flavonoids – Advances in research (J.B. Harborne and T.J. Mabry, eds), Chapman and Hall, London, 19-134.

Markham, K.R. and Geiger, H. (1994). ^{1}H-nmr spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulphoxide. In, The Flavonoids – Advances in research since 1986 (J.B. Harborne, ed), Chapman and Hall, London, 441-535.

Markham, K.R. and Given, D.R. (1988). The major flavonoids of an Antartic *Bryum*. *Phytochemistry*, *27*, 2843-2845.

Smith, H. (1972). In "Phytochrome" (K.Mitrakos and W. Shropshire, eds), Academic Press, N.Y. and London, 433.

Wolfender, J-L., Maillard, M., Marston, A. and Hostettman, K. (1992), Mass spectrometry of underivatised naturally occurring glycosides. *Phytochemical Analysis*, 3, 193-214.

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SPECTRA TABLES

(Página deixada propositadamente em branco)

TABLE I

Reference compounds grouped according to general compound type (e.g. aromatic acids, dihydroflavonoids, flavones, and flavonols)

N.º	1. Aromatic acids	RT	$\lambda_{max.}$ nm
1	. benzoic acid	31.5	265sh-272-280sh
2	. salicylic acid	34.7	301
3	. *p*-hydroxybenzoic acid	18.1	255
4	. 2,4-dihydroxybenzoic acid	22.6	240sh-296sh-325
5	. 2,5-dihydroxybenzoic acid	18.6	235sh-325
6	. vanillic acid	21.9	258-290sh
7	. gallic acid	8.9	270
8	. syringic acid	24.2	274
9	. cinnamic acid	41	276
10	. *o*-coumaric acid	36	233sh-273-320sh
11	. *m*-coumaric acid	33	233sh-274-320sh
12	. *p*-coumaric acid	28.6	293sh-308
13	. caffeic acid	23.5	239sh-296sh-323
14	. ferulic acid	31.4	239sh-296sh-323
15	. isoferulic acid	32.9	239sh-296sh-323
16	. sinapic acid	32.2	325
17	. chlorogenic acid	22.8	245sh-296sh-323
18	. rosmarinic acid	35	250sh-290sh-320
N.º	**2. Dihydroflavonoids**	**RT**	**$\lambda_{max.}$ nm**
19	. naringenin	44.9	289-326sh
20	. eriodictyol	40.8	289-324sh
21	. eriodictyol-7-*O*-[rhamnosyl(1-2)glucoside]	32.5	289-334sh
22	. fustin	29.8	278-311sh
23	. dihydroquercetin	32.8	288-334sh
N.º	**3. Flavones**	**RT**	**$\lambda_{max.}$ nm**
24	. 5-hydroxy-4'-methoxyflavone	59	253sh-324-366sh-392sh
25	. chrysin	56.3	266-312sh
26	. baicalein	53	277-321
27	. 5,7,2'-trihydroxyflavone	48.4	242sh-263-286sh-337

N.º	3. Flavones (cont.)	RT	$\lambda_{max.}$ nm
28	. apigenin	45.1	266-290sh-337
29	. apigenin-7-*O*-[rhamnosyl(1-2)glucoside]	35.7	267-339
30	. apiin (apigenin-7-*O*-[apiosyl(1-2) glucoside])	35.6	267-335
31	. apigenin-7-*O*-glucoside	35,8	267-336
32	. vitexin	32.6	266-290sh-336
33	. isovitexin	39.1	274-329
34	. 2''-*O*-glucosylvitexin	31.7	268-294sh-336
35	. saponarin	26.1	268-336
36	. genkwanin (7-*O*-methylapigenin)	57.1	267-290sh-331
37	. acacetin-7-*O*-[rhamnosyl(1-2)glucoside]	41.4	267-332
38	. 8-hydroxyapigenin-8-*O*-glucuronide	39.5	238sh- 270-295sh-330
39	. 8-hydroxyapigenin-7-*O*-allosyl(1-2) glucoside	35.2	274-303-326
40	. scutellarein-6,4'-dimethylether	58	274-332
41	. 5,7,2',5'-tetrahydroxyflavone	40.2	256sh-263-286sh-308-359
42	. luteolin	41.6	253-267sh-291sh-350
43	. luteolin-7-*O*-glucoside	33.6	254-267sh-348
44	. luteolin-3'-*O*-glucoside	36.5	240-268-288sh-342
45	. luteolin-5-*O*-glucoside	31.5	240sh-249-262sh-288sh-302sh-343
46	. luteolin-7-*O*-rutinoside	32.7	254-267sh-348
47	. luteolin-7-*O*-[glucuronosyl(1-2)-glucuronide]	29.8	255-267sh-345
48	. luteolin-7,4'-di-*O*-glucuronide	30.7	247sh-267sh-335
49	. luteolin-7,3'-di-*O*-glucuronide	33.1	240-268-340
50	luteolin-3',4'-di-*O*-glucuronide	34,5	271-291sh-323
51	. luteolin-7,3',4'-tri-*O*-glucuronide	28	272-293sh-321
52	. orientin	27.1	254-268sh-290sh-350
53	. isoorientin	27.0	254sh-268-290sh-350
54	. neocarlinoside (luteolin-6-*C*-glucoside-8-*C*-arabinoside)	28.9	256sh-271-294sh-350
55	. chrysoeriol	46.7	241sh-252-269-290sh-348
56	. chrysoeriol-5-*O*-glucoside	35	238sh-248sh-262sh-343
57	. chrysoeriol-7-*O*-glucuro-glucuronide	26.8	225-242sh-267-333
58	. chrysoeriol-6,8-di-*C*-glucoside	28.7	253sh-267-344

N.º	3. Flavones (cont.)	RT	$\lambda_{max.}$ nm
59	. diosmetin 7-*O*-[rhamnosyl(1-6)glucoside]	36.3	251-266sh-346
60	. 5,7-dihydroxy-2',5'-dimethoxyflavone	58.4	256-sh-265-310sh-355
61	. 6-hydroxyluteolin	35.7	251sh-278-310sh-344
62	. 6-hydroxyluteolin-7-*O*-[glucosyl(1-2) glucoside]	29.7	232sh-253sh-281-344
63	. 6-hydroxyluteolin-7-*O*-[xylosyl(1-2)glucoside]	38.7	232sh-245sh-253sh-282-345
64	. 6-hydroxyluteolin-7-*O*-[xylosyl(1-2) xyloside]	34.9	232sh-253sh-282-344
65	8-hydroxyluteolin-7-*O*-glucoside	29,6	255sh-277-298sh-340
66	8-hydroxyluteolin-8-*O*-glucoside	35,1	255sh-270-292sh-352
67	. tricetin	38	247sh-266-296sh-350
68	. tricetin-7-*O*-glucoside	30,7	247sh-266-350
69	. tricin	46.1	245-266-296sh-354
70	. tricin-5-*O*-glucoside	35	245-266-296sh-351
71	. tricin-7-*O*-[rhamnosyl(1-2)glucuronide]	36.2	248-267-296sh-351
72	. tricetin-3',4',5'-trimethylether	54.7	237sh-269-331
73	. scaposin	48.3	280-331
74	. 7-hydroxy-5,8,3',4'-tetramethoxyflavone	42.6	249-270-340

N.º	4. Flavonols	RT	$\lambda_{max.}$ nm
75	. 2'-hydroxyflavonol	48.8	240-292sh-330
76	. galangin	57.8	237sh-263-288sh-310-358
77	. 3',4'-dihydroxyflavonol	43.7	230sh-248-312sh-327sh-360
78	. 3',4'-dimethoxyflavonol	57.2	230sh-248-312sh-327sh-360
79	. 7,4'-di-*O*-methylflavonol	32.8	254-266sh-346
80	. 7,8,4'-trihydroxyflavonol	36.5	237sh-263-315-359
81	. 7,8,4'-trimethoxyflavonol	58	250sh-258-308sh-330
82	. fisetin	37.2	253-305-320sh-354
83	. kaempferol	46.6	248sh-266-295sh-318sh-366

N.º	4. Flavonols (cont.)	RT	$\lambda_{max.}$ nm
84	. kaempferol-3-*O*-glucoside	35.6	265-290sh-320sh-346
85	. kaempferol-3-*O*-rhamnoside	38.5	262-292sh-315sh-342
86	. kaempferol-7-O-glucoside	35.8	248sh-265-322sh-362
87	. kaempferol-3-*O*-[glucosyl(1-2)glucoside]	32.1	242sh-265-292sh-322sh-346
88	. kaempferol-3-*O*-[rhamnosyl(1-2)glucoside]	33.2	265-290sh-320sh-350
89	. kaempferol-3-*O*-[rhamnosyl(1-6)glucoside]	35.1	265-290sh-320sh-348
90	. kaempferol-3,7-*O*-diglucoside	27.4	242sh-265-322sh-345
91	. kaempferol-3-*O*-[rhamnosyl(1-6) glucoside]-7-*O*-rhamnoside	31.8	245sh-266-292sh-316sh-345
92	. kaempferol-7-*O*-glucoside-3-*O*-[glucosyl(1-2)glucoside]	23.7	242sh-263-315sh-344
93	. kaempferol-3-*O*-glucoside-7-*O*-rhamnoside	32	242sh-265-315sh-344
94	. kaempferol-3-*O*-[6-acetylglucoside]-7-*O*-glucoside	30.1	242sh-265-315sh-346
95	. kaempferol-3-*O*-[6-acetylglucoside]-7-*O*-rhamnoside	34.3	242sh-266-315sh-346
96	. kaempferol-3-*O*-[2-sinapoylglucosyl(1-2)glucoside]-7-*O* -glucoside	26.8	226sh-242sh-268-331
97	. kaempferol-4'-*O*-methylether	58.2	248sh-265-295sh-318sh-362
98	. 7-*O*-methylkaempferol-3-*O*-[rhamnosyl(1-2)glucoside]	32	242sh-263-315sh-344
99	. datiscetin	45.7	255-308sh-346
100	. datiscetin-3-*O*-[rhamnosyl(1-6)glucoside]	33.9	258-304sh-332sh
101	. herbacetin-8-*O*-glucoside	38.5	250sh-269-326sh-371
102	. herbacetin-3-*O*-glucosyl-8-*O*-glucuronide	29.8	269-295sh-326sh-352sh
103	. 8-*O*-methylherbacetin	46.3	250sh-272-325sh-375
104	. 8-*O*-methylherbacetin-3-*O*-[glucosyl(1-2)glucoside]	31.4	274-300sh-324sh-355sh
105	. 7-*O*-methylherbacetin	45.6	276-307sh-329-387
106	. 7-*O*-methylherbacetin-3-*O*-[glucosyl(1-2) glucoside]	30.9	278-307-330-370sh
107	. 7,8-di-*O*-methylherbacetin-3-*O*-[2-ferulylglucoside]	43	279-310sh-329
108	. quercetin	41.9	255-295sh-372
109	. quercetin-3-*O*-galactoside	33.5	255-266sh-294sh-355

N.º	4. Flavonols (cont.)	RT	$\lambda_{max.}$ nm
110 .	quercetin-3-*O*-glucoside	33.8	255-266sh-294sh-355
111 .	quercetin-3-*O*-arabinoside	34.9	255-266sh-294sh-354
112 .	quercetin-3-*O*-rhamnoside	35.7	254-263sh-294sh-348
113 .	quercetin-7-*O*-glucoside	32.6	254-269sh-370
114 .	quercetin-3-*O*-[glucosyl(1-2)glucoside]	30.6	255-266sh-294sh-355
115 .	quercetin-3-*O*-[rhamnosyl(1-6) galactoside]	32.7	255-266sh-294sh-355
116 .	quercetin-3-*O*-[rhamnosyl(1-6)glucoside]	33.1	255-264sh-294sh-355
117 .	3-*O*-methylquercetin	43.4	255-263sh-297sh-357
118 .	isorhamnetin	47.3	255-266sh-306sh-326sh-372
119 .	isorhamnetin-3-*O*-galactoside	36	253-266sh-294sh-352
120 .	isorhamnetin-3-*O*-glucoside	36.3	255-266sh-294sh-355
121 .	isorhamnetin-3-*O*-[rhamnosyl(1-6) glucoside]	35.5	255-266sh-294sh-355
122 .	tamarixetin-7-*O*-rutinoside	35.9	253-269sh-369
123 .	5-*O*-methylisorhamnetin-3-*O*-glucoside	33.8	249-263sh-298sh-344
124 .	penta-*O*-methylquercetin	55.5	248-262sh-318sh-346
125 .	morin	39.5	252-262sh-290sh-318sh-352
126 .	patuletin-7-*O*-glucoside	33.5	256-272sh-372
127 .	quercetagenin-7-*O*-glucoside	30.7	236sh-259-274sh-358
128 .	gossypetin-7-*O*-glucoside	31.8	259-269sh-300sh-340sh-381
129 .	gossypetin-8-*O*-glucoside-3-*O*-[glucosyl (1-2) glucoside]	24.6	259sh-268-356
130 .	robinetin (5-deoxymyricetin)	33,7	246-316-358
131 .	3-*O*-methyl-7,8,3',4'-tetrahydroxyflavonol	33.2	255-265sh-310sh-345
132 .	myricetin	37.2	252-263sh-298sh-371
133 .	myricetin-3-*O*-galactoside	30.8	250-261sh-300sh-354
134	myricetin-3-*O*-rhamnoside	32.8	250sh-261-300sh-349

TABLE II

Reference compounds listed in order of retention times (RT)

RT	N.º	Compound	$\lambda_{max.}$ nm
8,9	7	gallic acid	270
18,1	3	*p*-hydroxybenzoic acid	255
18,6	5	2,5-dihydroxybenzoic acid	235sh-325
21,9	6	vanillic acid	258-290sh
22,6	4	2,4-dihydroxybenzoic acid	240sh-296sh-325
22,8	17	chlorogenic acid	245sh-296sh-323
23,5	13	caffeic acid	239sh-296sh-323
23,7	92	kaempferol-7-*O*-glucoside-3-*O*-[glucosyl(1-2)glucoside]	242sh-263-315sh-344
24,2	8	syringic acid	274
24,6	129	gossypetin-8-*O*-glucoside-3-*O*-[glucosyl (1-2)glucoside]	259sh-268-356
26,1	35	saponarin	268-336
26,8	96	kaempferol-3-*O*-[2-sinapoylglucosyl(1-2)glucoside]-7-*O* -glucoside	226sh-242sh-268-331
26,8	57	chrysoeriol-7-*O*-glucuro-glucuronide	225-242sh-267-333
27,0	53	isoorientin	254sh-268-290sh-350
27,1	52	orientin	254-268sh-290sh-350
27,4	90	kaempferol-3,7-*O*-diglucoside	242sh-265-322sh-345
28	51	luteolin-7,3',4'-tri-*O*-glucuronide	272-293sh-321
28,6	12	*p*-coumaric acid	293sh-308
28,7	58	chrysoeriol-6,8-di-*C*-glucoside	253sh-267-344
28,9	54	neocarlinoside (luteolin-6-*C*-glucoside-8-*C*-arabinoside)	256sh-271-294sh-350
29,6	65	8-hydroxyluteolin-7-*O*-glucoside	255sh-277-298sh-340
29,7	62	6-hydroxyluteolin-7-*O*-[glucosyl(1-2) glucoside]	232sh-253sh-281-344
29,8	22	fustin	278-311sh
29,8	47	luteolin-7-*O*-[glucuronosyl(1-2)-glucuronide]	255-267sh-345
29,8	102	herbacetin-3-*O*-glucosyl-8-*O*-glucuronide	269-295sh-326sh-352sh
30,1	94	kaempferol-3-*O*-[6-acetylglucoside]-7-*O*-glucoside	242sh-265-315sh-346
30,6	114	quercetin-3-*O*-[glucosyl(1-2)glucoside]	255-266sh-294sh-355
30,7	68	tricetin-7-*O*-glucoside	247sh-266-350

RT	N.º	Compound	$\lambda_{max.}$ nm
30,7	127	quercetagenin-7-*O*-glucoside	236sh-259-274sh-358
30.7	48	luteolin-7,4'-di-*O*-glucuronide	247sh-267sh-335
30,8	133	myricetin-3-*O*-galactoside	250-261sh-300sh-354
30,9	106	7-*O*-methylherbacetin-3-*O*-[glucosyl(1-2) glucoside]	278-307-330-370sh
31,4	14	ferulic acid	239sh-296sh-323
31,4	104	8-*O*-methylherbacetin-3-*O*-[glucosyl(1-2)glucoside]	274-300sh-324-355sh
31,5	1	benzoic acid	265sh-272-280sh
31,5	45	luteolin-5-*O*-glucoside	240sh-249-262sh-288sh-302sh-343
31,7	34	2''-*O*-glucosylvitexin	268-294sh-336
31,8	91	kaempferol-3-*O*-[rhamnosyl(1-6) glucoside]-7-*O*-rhamnoside	245sh-266-292sh-316sh-345
31,8	128	gossypetin-7-*O*-glucoside	259-269sh-300sh-340sh-381
32	93	kaempferol-3-*O*-glucoside-7-*O*-rhamnoside	242sh-265-315sh-344
32	98	7-*O*-methylkaempferol-3-*O*-[rhamnosyl(1-2)glucoside]	242sh-263-315sh-344
32,1	87	kaempferol-3-*O*-[glucosyl(1-2)glucoside]	242sh-265-292sh-322sh-346
32,2	16	sinapic acid	325
32,5	21	eriodictyol-7-*O*-[rhamnosyl(1-2)glucoside]	289-334sh
32,6	32	vitexin	266-290sh-336
32,6	113	quercetin-7-*O*-glucoside	254-269sh-370
32,7	46	luteolin-7-*O*-rutinoside	254-267sh-348
32,7	115	quercetin-3-*O*-[rhamnosyl(1-6) galactoside]	255-266sh-294sh-355
32,8	23	dihydroquercetin	288-334sh
32,8	79	7,4'-di-*O*-methylflavonol	254-266sh-346
32,8	134	myricetin-3-*O*-rhamnoside	250sh-261-300sh-349
32,9	15	isoferulic acid	239sh-296sh-323
33	11	*m*-coumaric acid	233sh -274-320sh
33,1	49	luteolin-7,3'-di-*O*-glucuronide	240-268-340
33,1	116	quercetin-3-*O*-[rhamnosyl(1-6)glucoside]	255-264sh-294sh-355

RT	N.º	Compound	$\lambda_{max.}$ nm
33,2	88	kaempferol-3-*O*-[rhamnosyl(1-2)glucoside]	265-290sh-320sh-350
33,2	131	3-*O*-methyl-7,8,3',4'-tetrahydroxyflavonol	255-265sh-310sh-345
33,5	109	quercetin-3-*O*-galactoside	255-266sh-294sh-355
33,5	126	patuletin-7-*O*-glucoside	256-272sh-372
33,6	43	luteolin-7-*O*-glucoside	254-267sh-348
33,7	130	robinetin (5-deoxymyricetin)	246-316-358
33,8	110	quercetin-3-*O*-glucoside	255-266sh-294sh-355
33,8	123	5-*O*-methylisorhamnetin-3-*O*-glucoside	249-263sh-298sh-344
33,9	100	datiscetin-3-*O*-[rhamnosyl(1-6)glucoside]	258-304sh-332sh
34,3	95	kaempferol-3-*O*-[6-acetylglucoside]-7-*O*-rhamnoside	242sh-266-315sh-346
34,5	50	luteolin-3',4'-di-*O*-glucuronide	271- 291sh-323
34,7	2	salicylic acid	301
34,9	64	6-hydroxyluteolin-7-*O*-[xylosyl(1-2) xyloside]	232sh-253sh-282-344
34,9	111	quercetin-3-*O*-arabinoside	255-266sh-294sh-354
35	18	rosmarinic acid	250sh-290sh-320
35	56	chrysoeriol-5-*O*-glucoside	238sh-248sh-262sh-343
35	70	tricin-5-*O*-glucoside	245-266-296sh -351
35,1	66	8-hydroxyluteolin-8-*O*-glucoside	255sh-270-292sh-352
35,1	89	kaempferol-3-*O*-[rhamnosyl(1-6)glucoside]	265-290sh-320sh-348
35,2	39	8-hydroxyapigenin-7-*O*-allosyl(1-2) glucoside	274-303-326
35,5	121	isorhamnetin-3-*O*-[rhamnosyl(1-6) glucoside]	255-266sh-294sh-355
35,6	30	apiin (apigenin-7-*O*-[apiosyl(1-2) glucoside])	267-335
35,6	84	kaempferol-3-*O*-glucoside	265-290sh-320sh-346
35,7	29	apigenin-7-*O*-[rhamnosyl(1-2)glucoside]	267-339
35,7	61	6-hydroxyluteolin	251sh-278-310sh-344
35,7	112	quercetin-3-*O*-rhamnoside	254-263sh-294sh-348
35,8	31	apigenin-7-*O*-glucoside	267-336

RT	N.º	Compound	$\lambda_{max.}$ nm
35,8	86	kaempferol-7-*O*-glucoside	248sh-265-322sh-362
35,9	122	tamarixetin-7-*O*-rutinoside	253-269sh-369
36	10	*o*-coumaric acid	233sh-273-320sh
36	119	isorhamnetin-3-*O*-galactoside	253-266sh-294sh-352
36,2	71	tricin-7-*O*-[rhamnosyl (1-2)glucuronide]	248-267-296sh-351
36,3	59	diosmetin 7-*O*-[rhamnosyl (1-6)glucoside]	251-266sh-346
36,3	120	isorhamnetin-3-*O*-glucoside	255-266sh-294sh-355
36,5	44	luteolin-3'-*O*-glucoside	240-268-288sh-342
36,5	80	7,8,4'-trihydroxyflavonol	237sh-263-315-359
37,2	82	fisetin	253-305-320sh-354
37,2	132	myricetin	252-263sh-298sh-371
38	67	tricetin	247sh-266-296sh-350
38,5	101	herbacetin-8-*O*-glucoside	250sh-269-326sh-371
38,5	85	kaempferol-3-*O*-rhamnoside	262-292sh-315sh-342
38,7	63	6-hydroxyluteolin-7-*O*-[xylosyl(1-2)glucoside]	232sh-245sh-253sh-282-345
39,1	33	isovitexin	274-329
39,5	38	8-hydroxyapigenin-8-*O*-glucuronide	238sh-270-295sh-330
39,5	125	morin	252-262sh-290sh-318sh-352
40,2	41	5,7,2',5'-tetrahydroxyflavone	256sh-263-286sh-308-359
40,8	20	eriodictyol	289-324sh
41	9	cinnamic acid	276
41,4	37	acacetin-7-*O*-[rhamnosyl(1-2)glucoside]	267-332
41,6	42	luteolin	253-267sh-291sh-350
41,9	108	quercetin	255-295sh-372
42,6	74	7-hydroxy-5,8,3',4'-tetramethoxyflavone	249-270-340
43	107	7,8-di-*O*-methylherbacetin-3-*O*-[2-ferulylglucoside]	279-310sh-329
43,4	117	3-*O*-methylquercetin	255-263sh-297sh-357
43,7	77	3',4'-dihydroxyflavonol	230sh-248-312sh-327sh-360
44,9	19	naringenin	289-326sh

RT	N.º	Compound	$\lambda_{max.}$ nm
45,1	28	. apigenin	266-290sh-337
45,6	105	. 7-*O*-methylherbacetin	276-307sh-329-387
45,7	99	. datiscetin	255-308sh-346
46,1	69	. tricin	245-266-296sh –354
46,3	103	. 8-*O*-methylherbacetin	250sh-272-325sh-375
46,6	83	. kaempferol	248sh-266-295sh-318sh-366
46,7	55	. chrysoeriol	241sh-252-269-290sh-348
47,3	118	. isorhamnetin	255-266sh-306sh-326sh-372
48,3	73	. scaposin	280-331
48,4	27	. 5,7,2'-trihydroxyflavone	242sh-263-286sh-337
48,8	75	. 2'-hydroxyflavonol	240-292sh-330
53	26	. baicalein	277-321
54,7	72	. tricetin-3',4',5'-trimethylether	237sh-269-331
55,5	124	. penta-*O*-methylquercetin	248-262sh-318sh-346
56,3	25	. chrysin	266-312sh
57,1	36	. genkwanin(7-*O*-methylapigenin)	267-290sh-331
57,2	78	. 3',4'-dimethoxyflavonol	230sh-248-312sh-327sh-360
57,8	76	. galangin	237sh-263-288sh-310-358
58	40	. scutellarein-6,4'-dimethylether	274-332
58	81	. 7,8,4'-trimethoxyflavonol	250sh-258-308sh-330
58,2	97	. kaempferol-4'-*O*-methylether	248sh-265-295sh-318sh-362
58,4	60	. 5,7-dihydroxy-2',5'-dimethoxyflavone	256-sh-265-310sh-355
59	24	. 5-hydroxy-4'-methoxyflavone	253sh-324-366sh-392sh

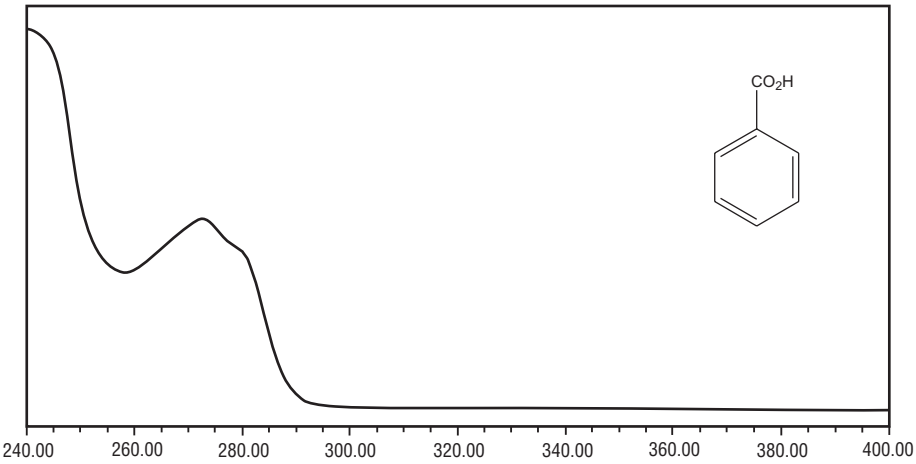
UV-SPECTRA

(Página deixada propositadamente em branco)

1. PHENOLIC ACIDS

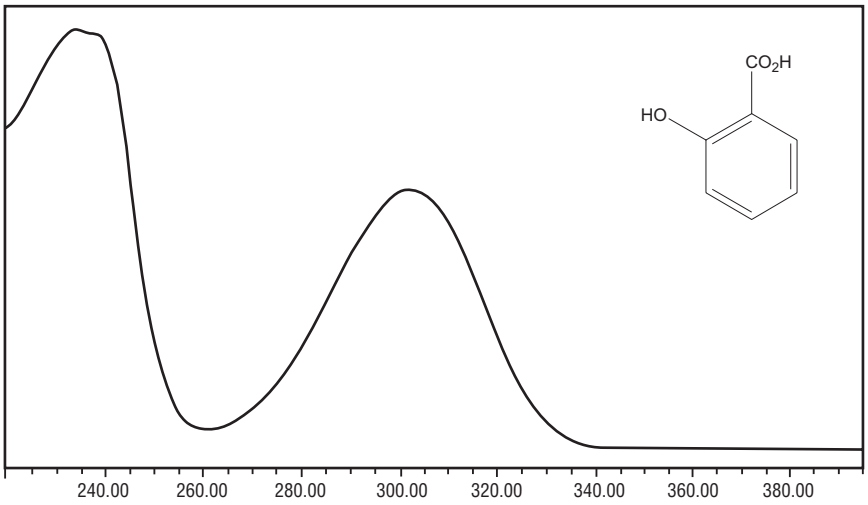
1 – benzoic acid

(RT= 31.5; $\lambda_{max.}$= 265sh, 272, 280sh nm)



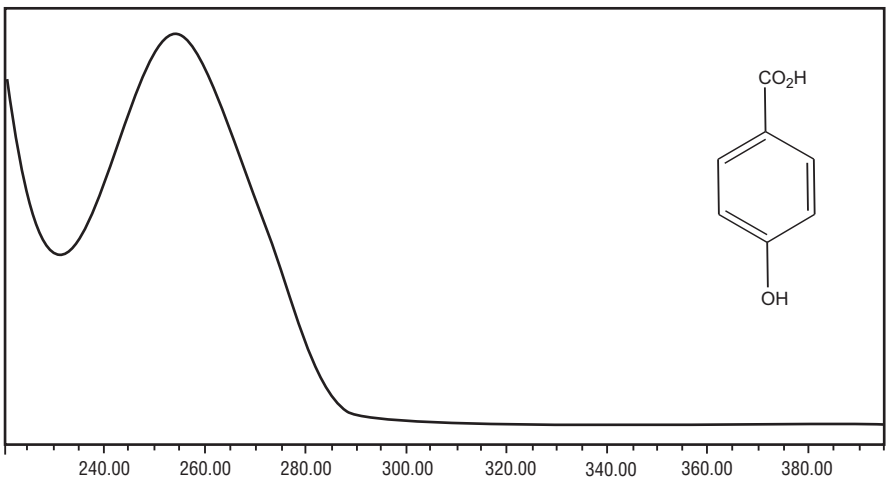
2 – salicylic acid

(RT= 34.7; $\lambda_{max.}$= 301 nm)



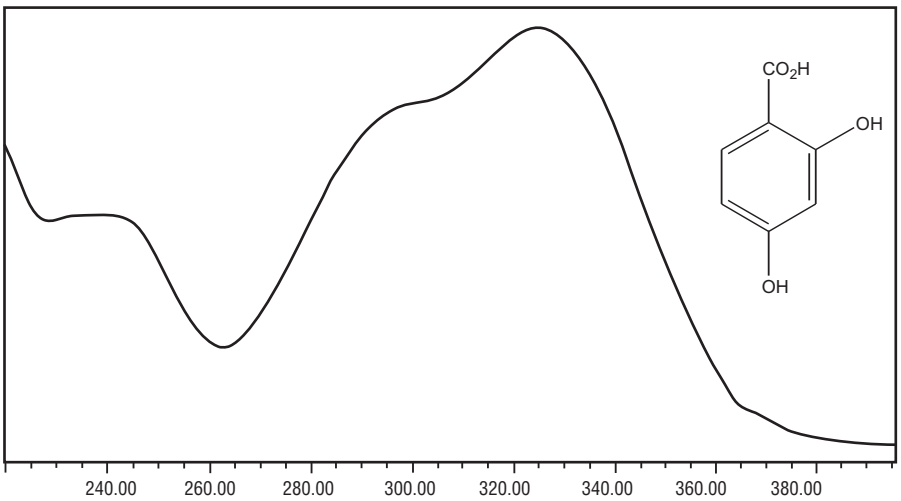
3 – *p*-hydroxybenzoic acid

(RT= 18.1; $\lambda_{max.}$= 255 nm)



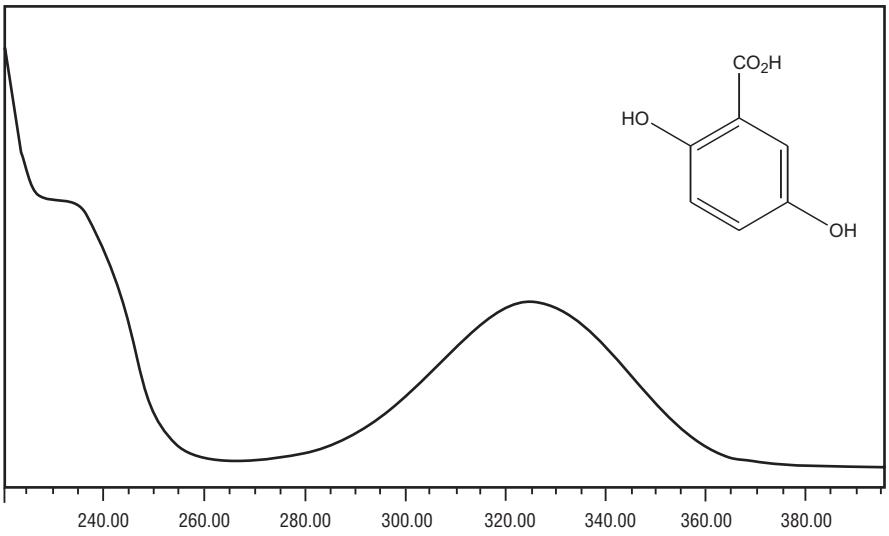
4 – 2,4-dihydroxybenzoic acid

(RT= 22.6; $\lambda_{max.}$= 240sh, 296sh, 325 nm)



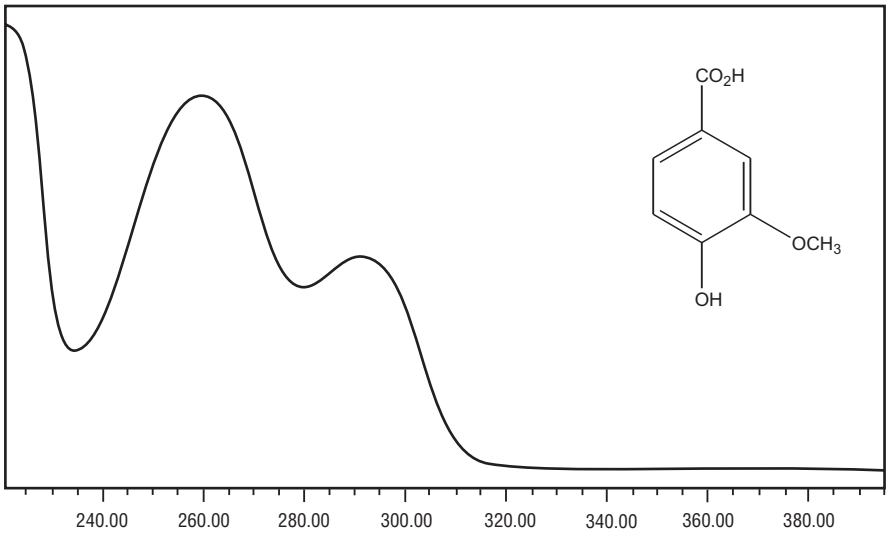
5 – 2,5-dihydroxybenzoic acid

(RT=18.6; $\lambda_{max.}$= 235sh, 325 nm)



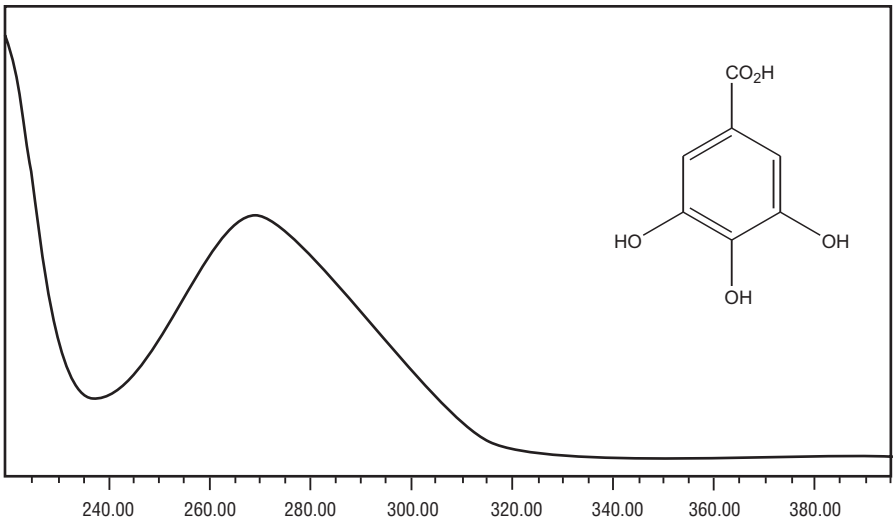
6 – vanillic acid

(RT= 21.9; $\lambda_{max.}$= 258, 290sh nm)



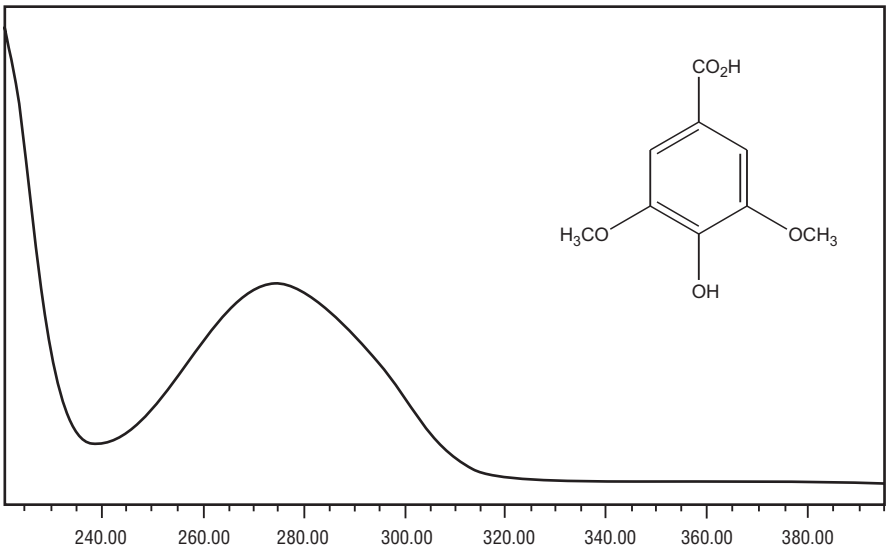
7 – gallic acid

(RT= 8.9; $\lambda_{max.}$= 270 nm)



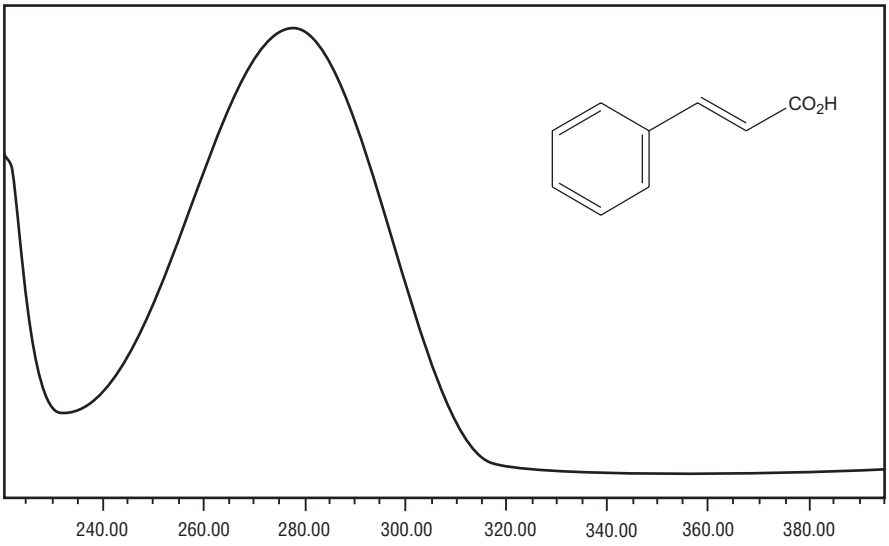
8 – syringic acid

(RT= 24,2; $\lambda_{max.}$= 274 nm)



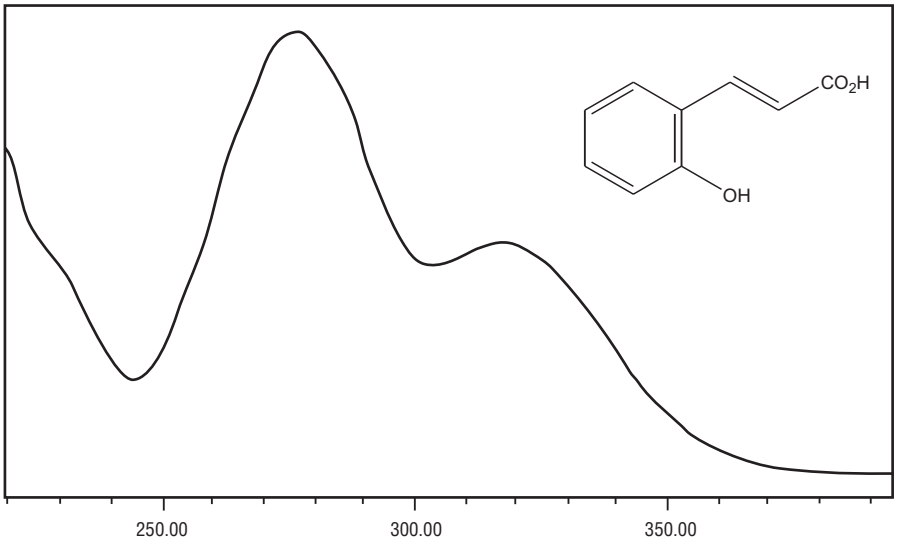
9 – cinnamic acid

(RT= 41; $\lambda_{max.}$= 276 nm)



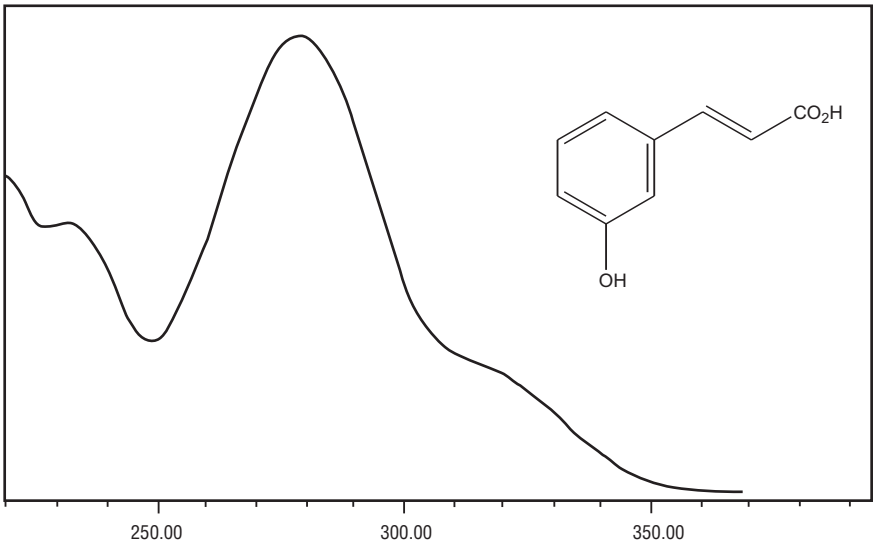
10 – *o*-coumaric acid

(RT= 36; λ_{max} = 233sh, 273, 320sh nm)



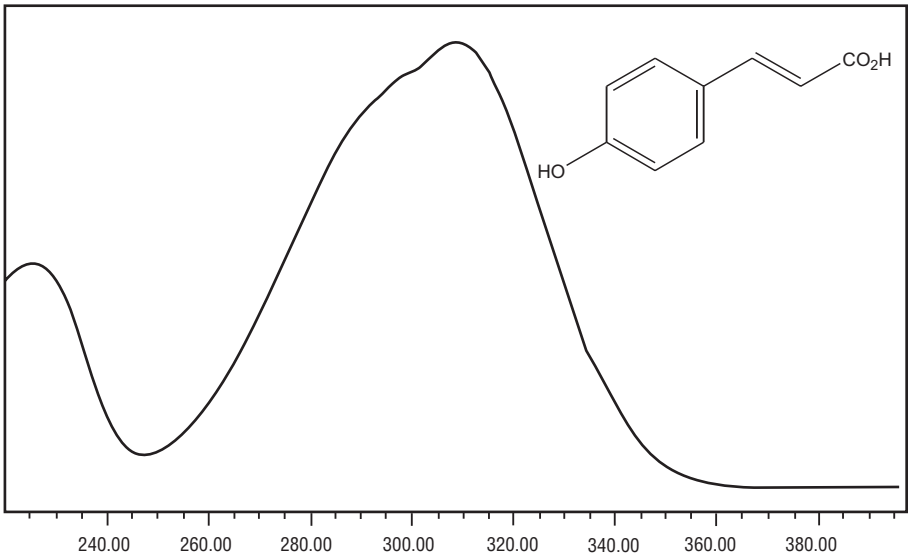
11 – *m*-coumaric acid

(RT= 33; λ_{max} = 233sh, 274, 320sh nm)



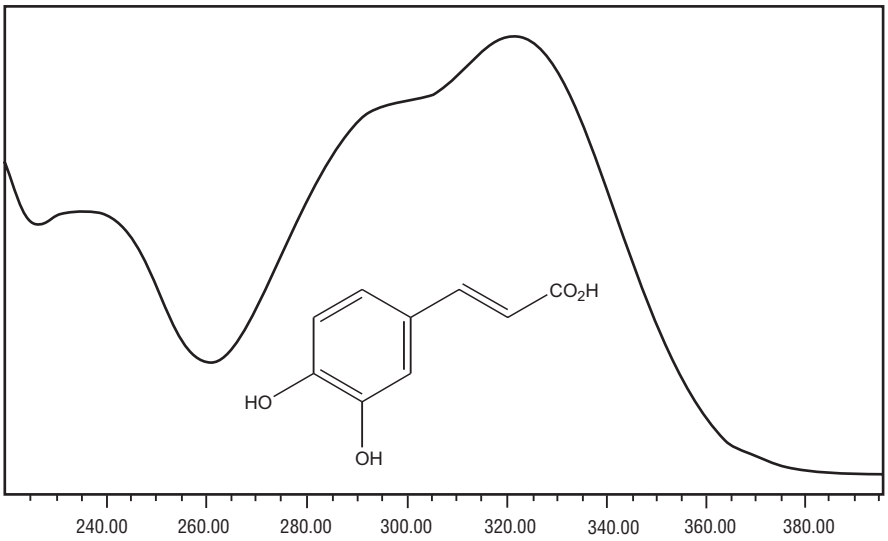
12 – *p*-coumaric acid

(RT= 28.6; $\lambda_{max.}$= 293sh, 308 nm)



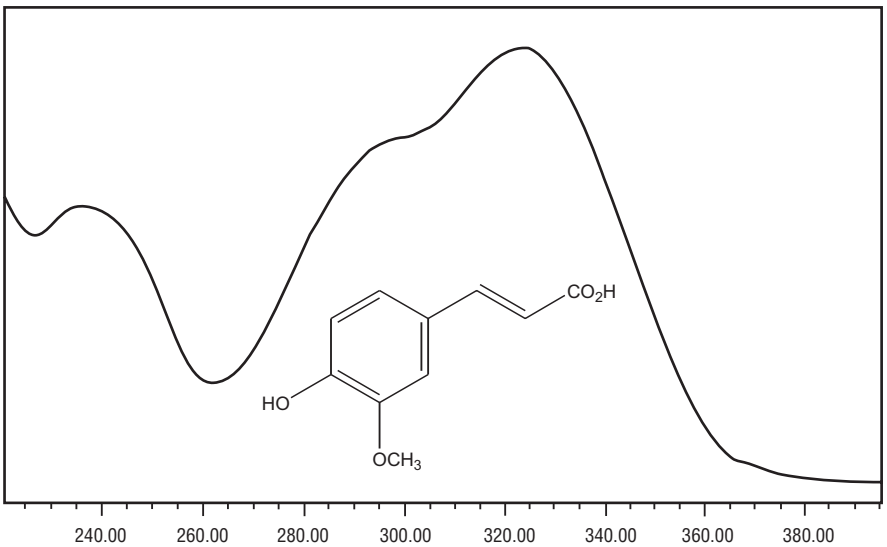
13 – caffeic acid

(RT= 23.5; $\lambda_{max.}$= 239sh, 296sh, 323 nm)



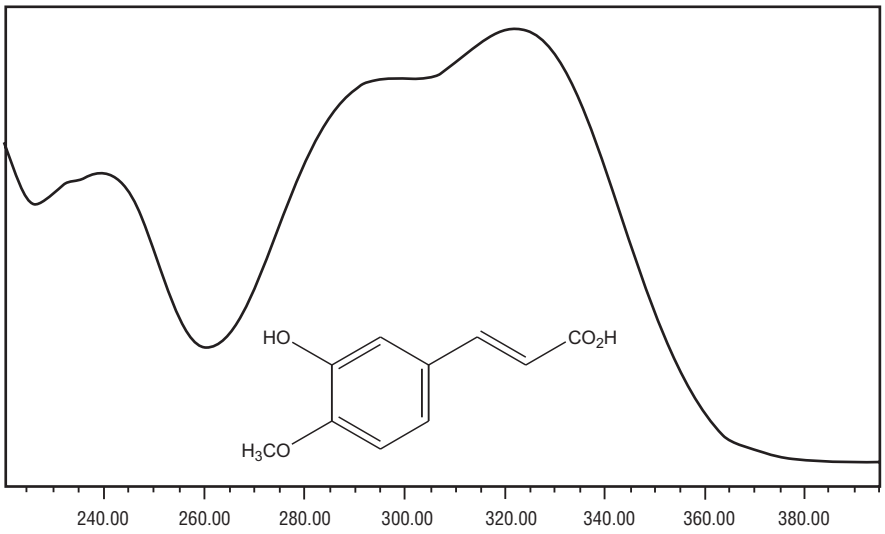
14 – ferulic acid

(RT= 31.4; $\lambda_{max.}$= 239sh, 296sh, 323 nm)



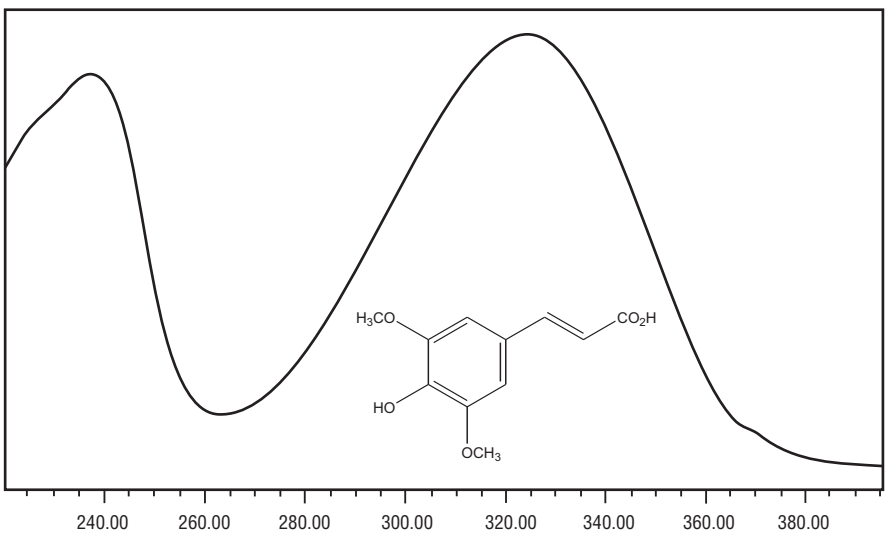
15 – isoferulic acid

(RT= 32.9; $\lambda_{max.}$ = 239sh, 296sh, 323 nm)



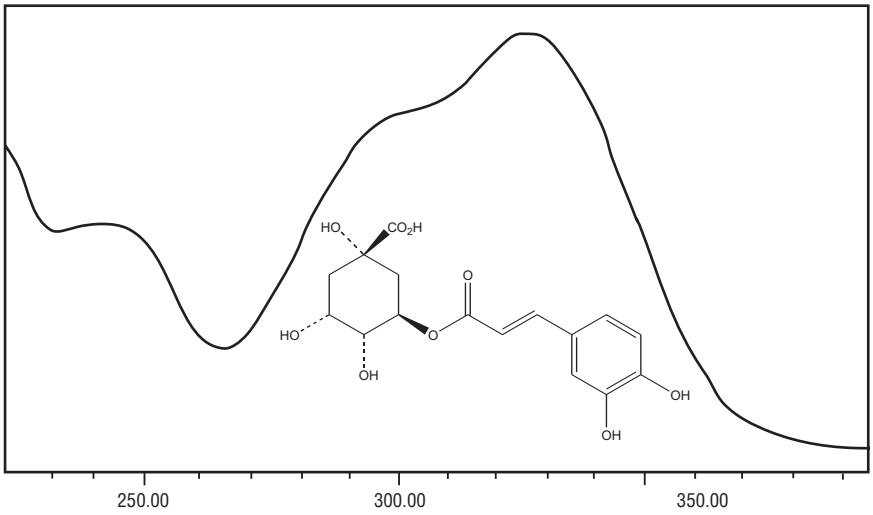
16 – sinapic acid

(RT= 32.2; $\lambda_{max.}$ = 325 nm)



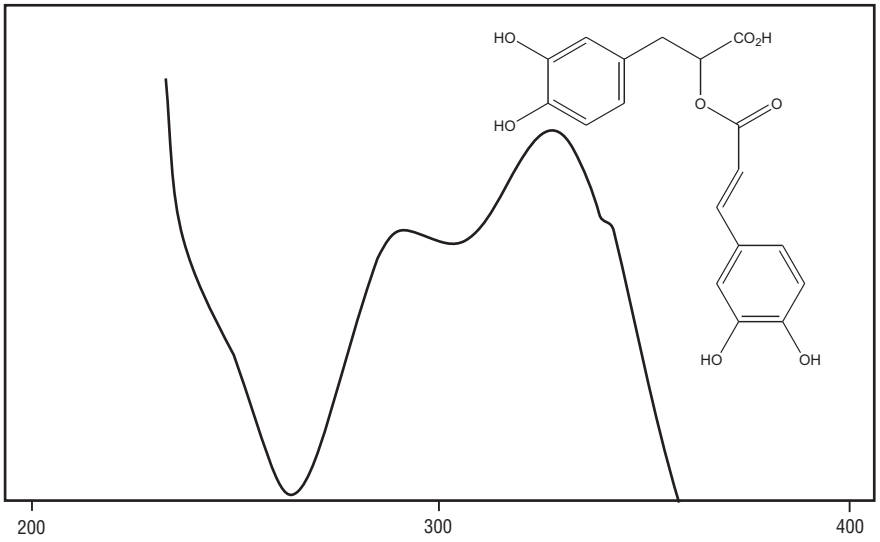
17 – chlorogenic acid

(RT= 22.8; $\lambda_{max.}$= 245sh, 296sh, 323 nm)



18 – rosmarinic acid

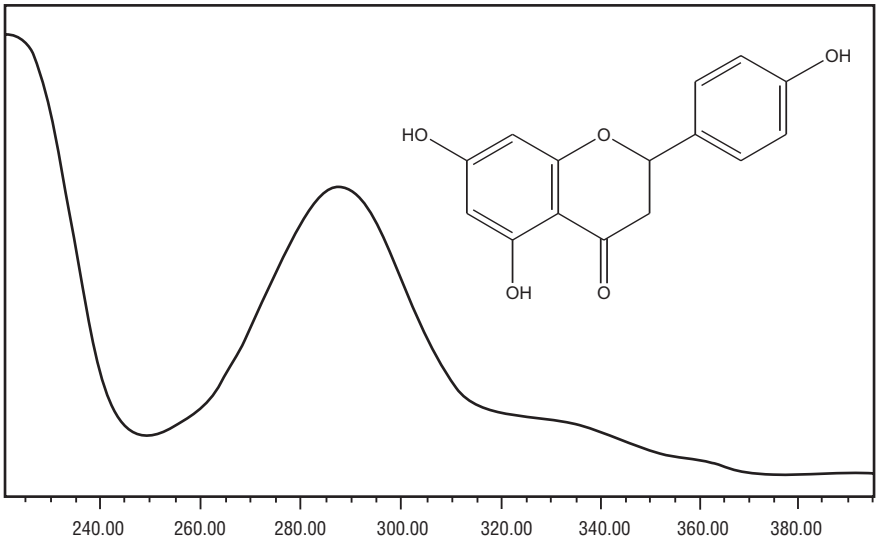
(RT= 35; $\lambda_{max.}$= 250sh, 290sh, 320 nm)



2. DIHYDROFLAVONOIDS

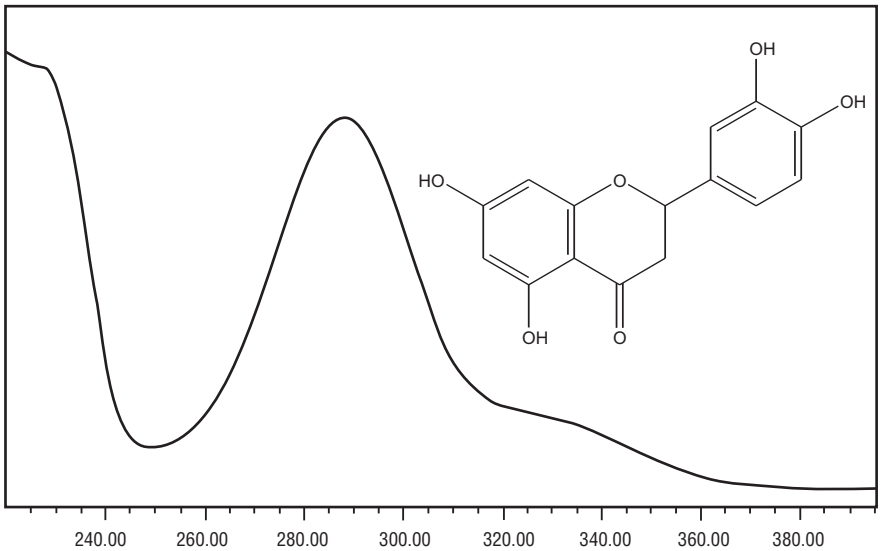
19 – naringenin

(RT= 44.9; $\lambda_{max.}$= 289, 326sh nm)



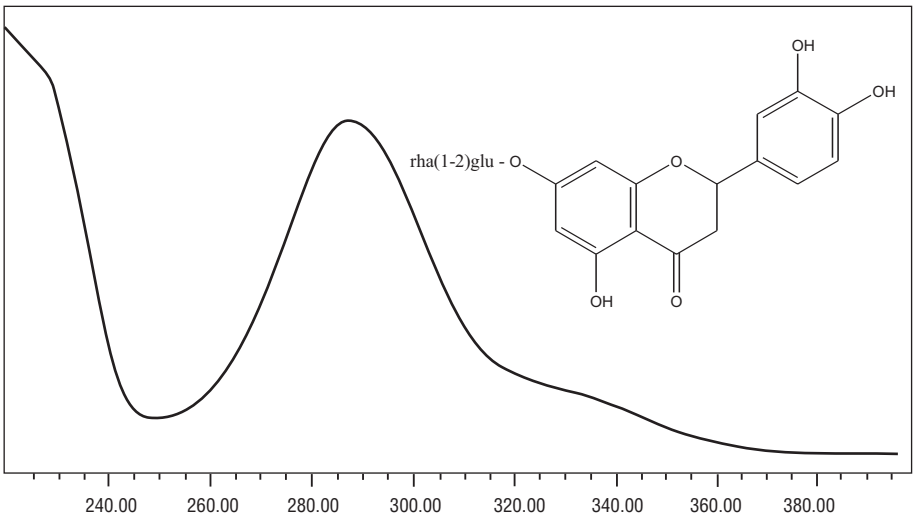
20 – eridioctyol

(RT= 40.8; $\lambda_{max.}$= 289, 324sh nm)



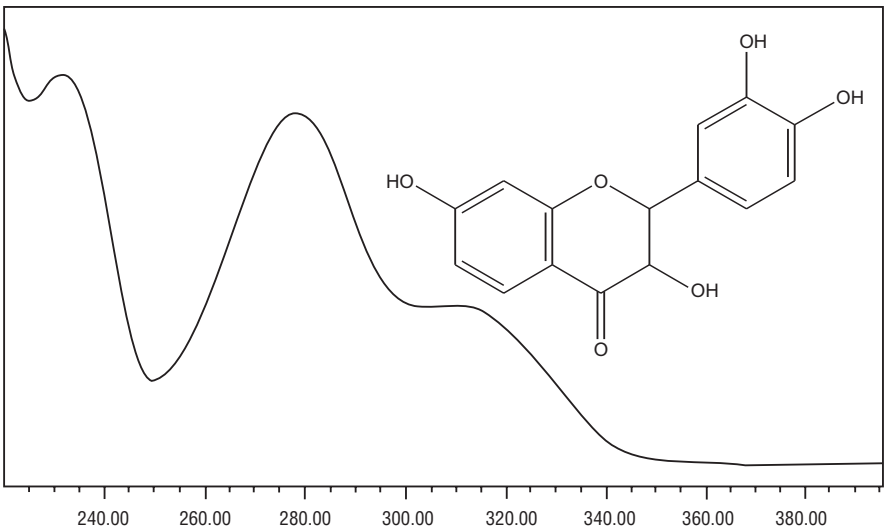
21 – **eriodictyol-7-*O*-[rhamnosyl(1-2)glucoside]**

(RT= 32.5; $\lambda_{max.}$= 289, 334sh nm)



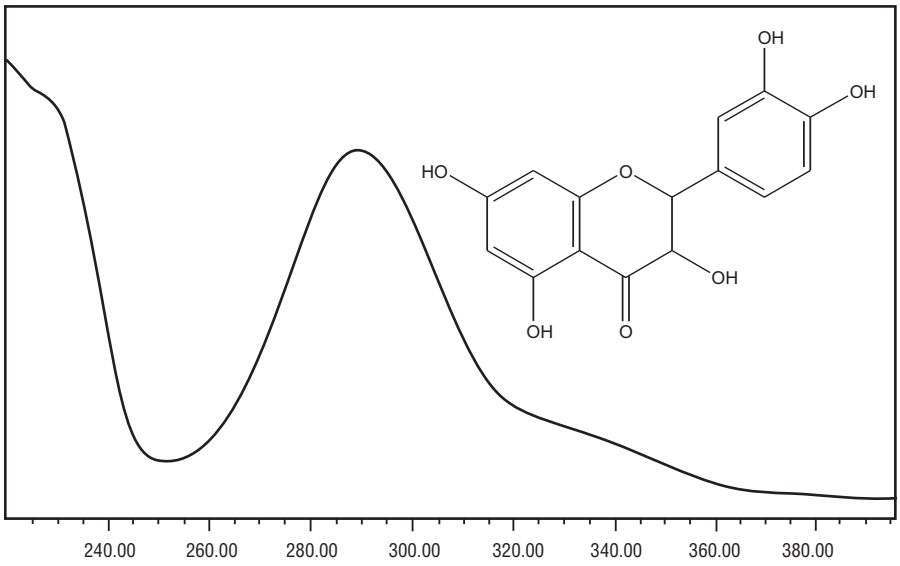
22 – **fustin**

(RT= 29.8; $\lambda_{max.}$= 233, 278, 311sh nm)



23 – **dihydroquercetin**

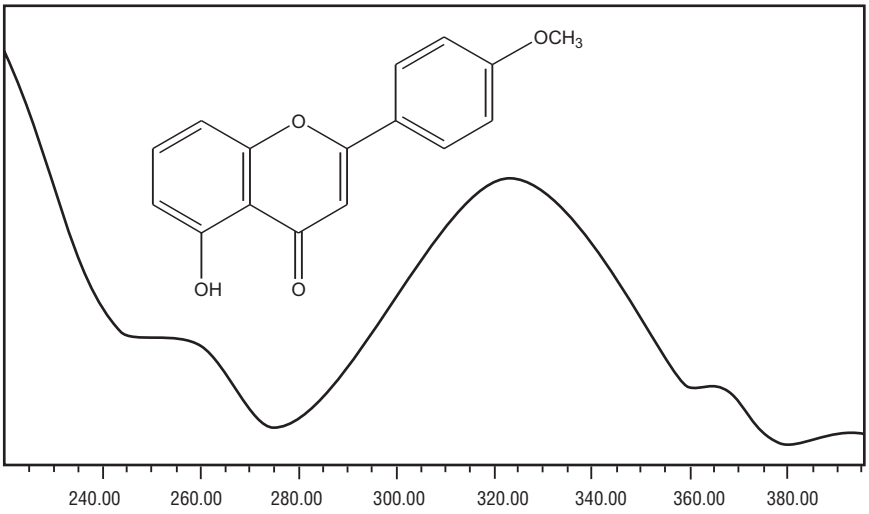
(RT= 32.8; $\lambda_{max.}$= 288, 334 nm)



3. FLAVONES

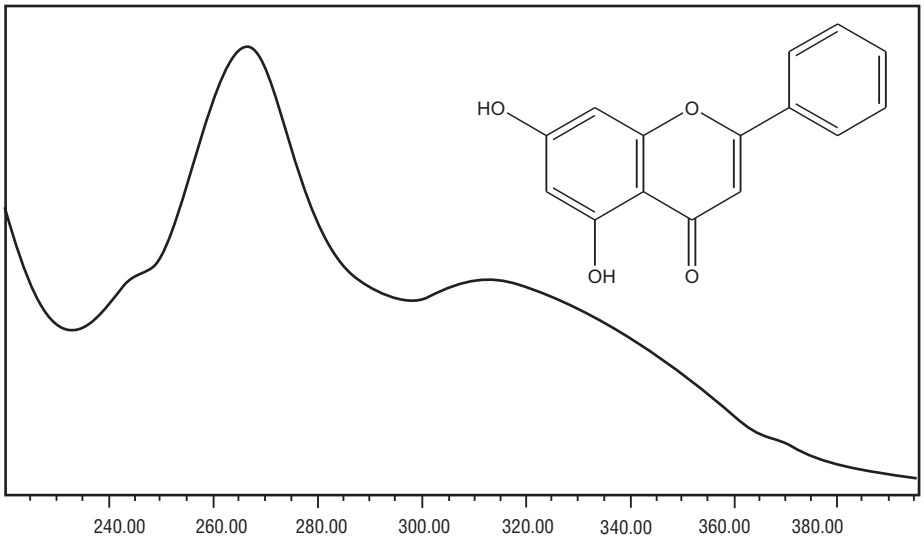
24 – 5-hydroxy-4'-methoxyflavone

(RT = 59; $\lambda_{max.}$ = 253sh, 324, 366sh, 392sh nm)



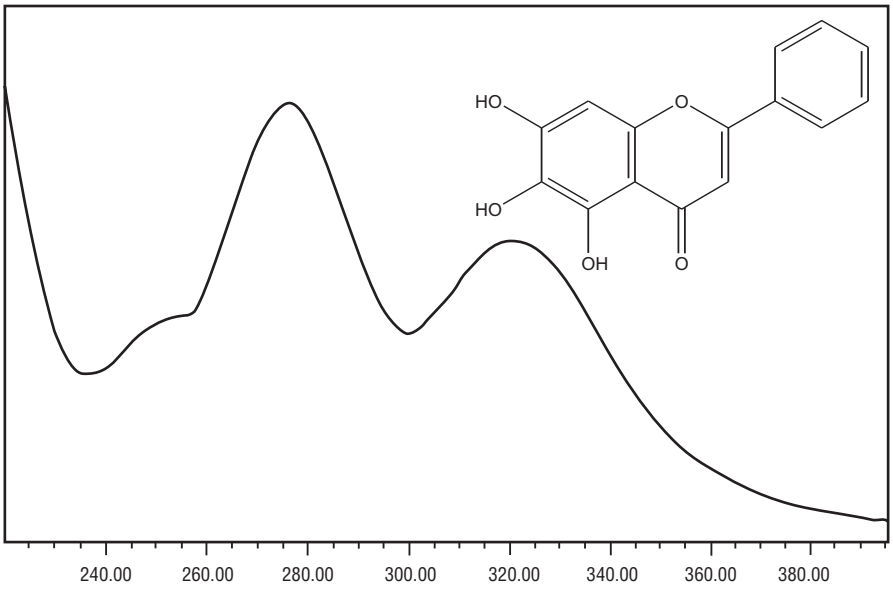
25 – chrysin

(RT = 56.3; $\lambda_{max.}$ = 266, 312sh nm)



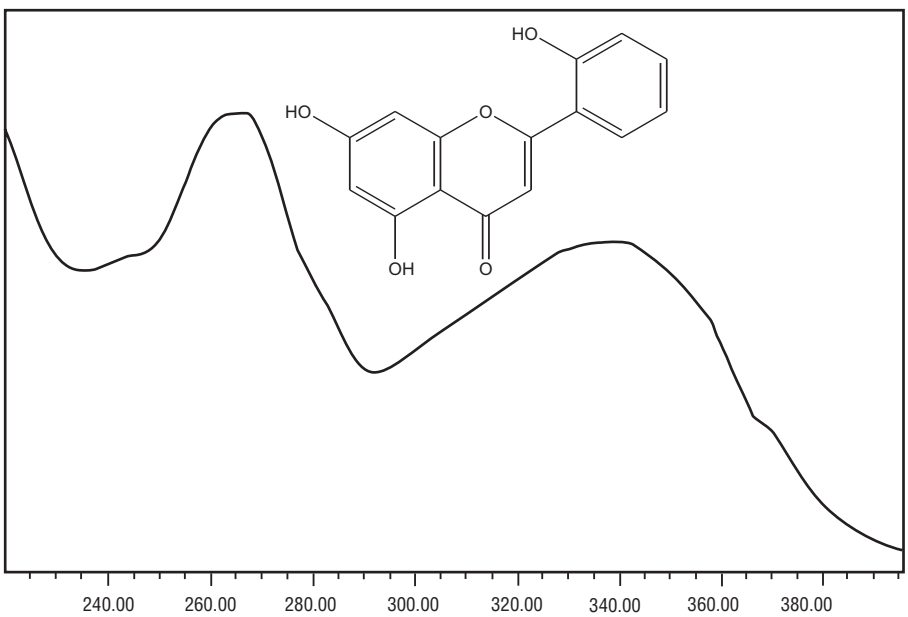
26 – **baicalein**

(RT = 53; $\lambda_{max.}$ = 277, 321 nm)



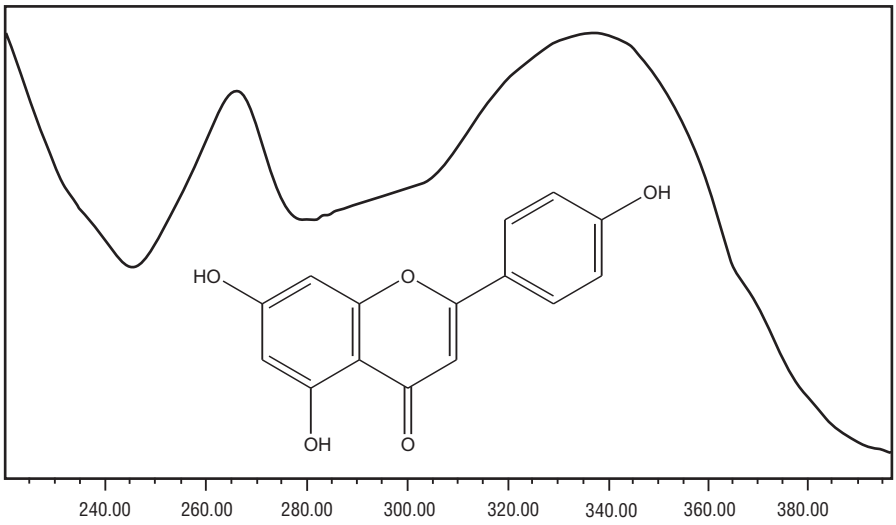
27 – **5,7,2'-trihydroxyflavone**

(RT = 48.4 ; $\lambda_{max.}$ = 242sh, 263, 286sh, 337 nm)



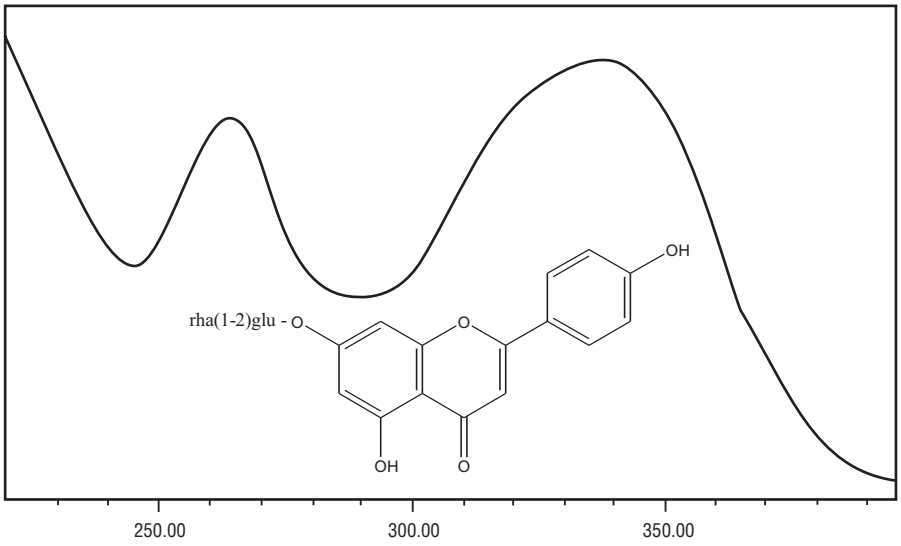
28 – apigenin

(RT = 45.1; $\lambda_{max.}$ = 266, 290sh, 337 nm)

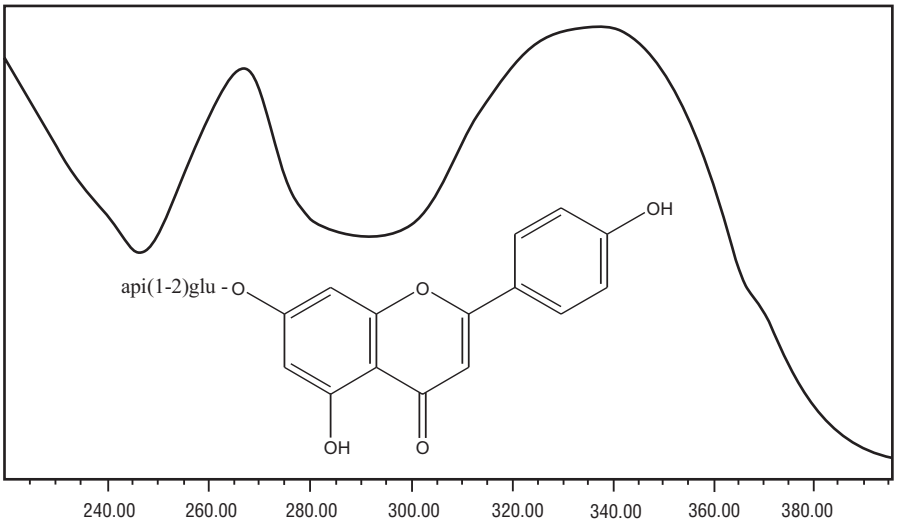


29 – apigenin-7-*O*-[rhamnosyl(1-2)glucoside]

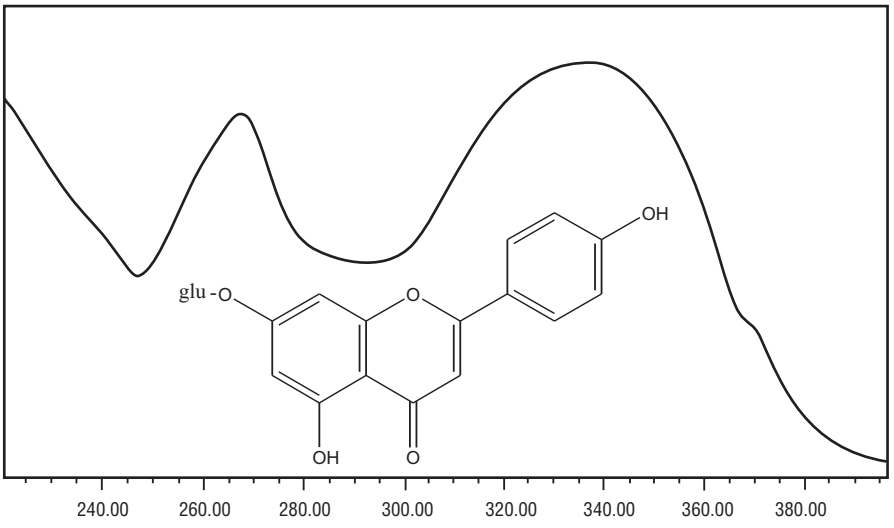
(RT = 35.7; $\lambda_{max.}$ = 267, 339 nm)



30 – apigenin-7-*O*-[apiosyl(1-2)glucoside]
(RT = 35.6; $\lambda_{max.}$ = 267, 335 nm)

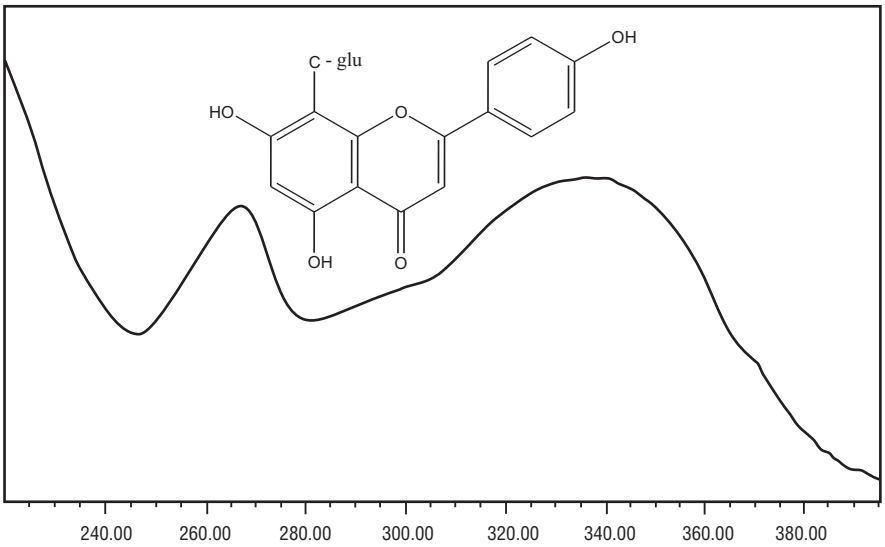


31 – apigenin-7-*O*-glucoside
(RT = 35.8; $\lambda_{max.}$ = 267, 336 nm)



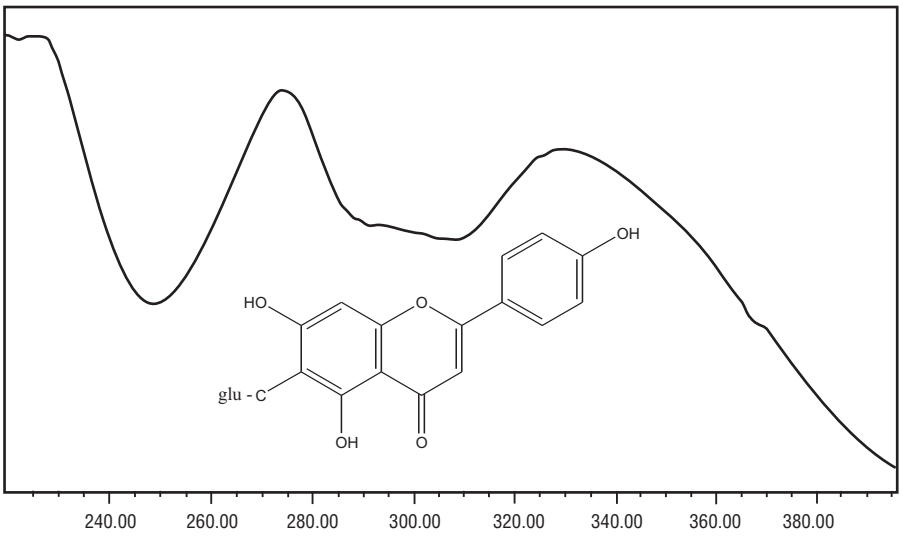
32 – **vitexin**

(RT = 32.6; $\lambda_{max.}$ = 266, 290sh, 336 nm)



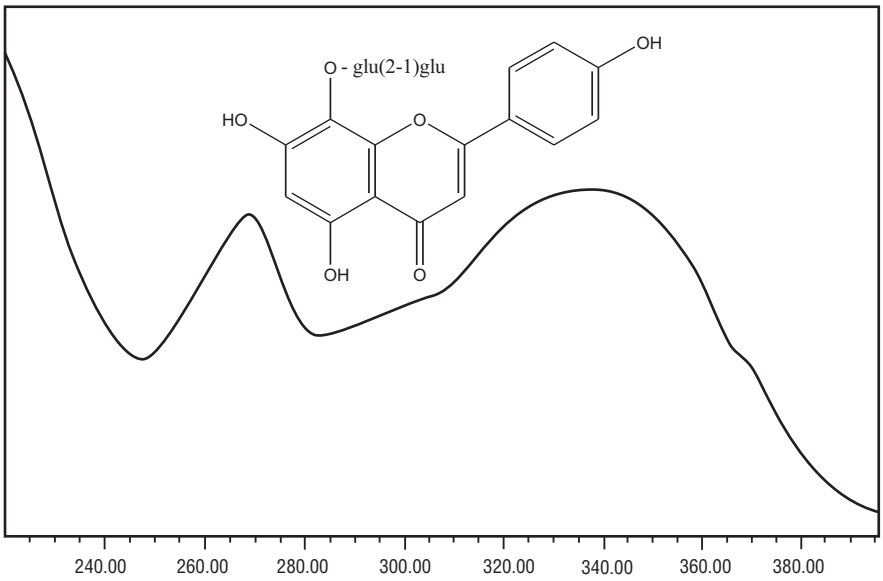
33 – **isovitexin**

(RT = 39.1; $\lambda_{max.}$ = 274, 329 nm)



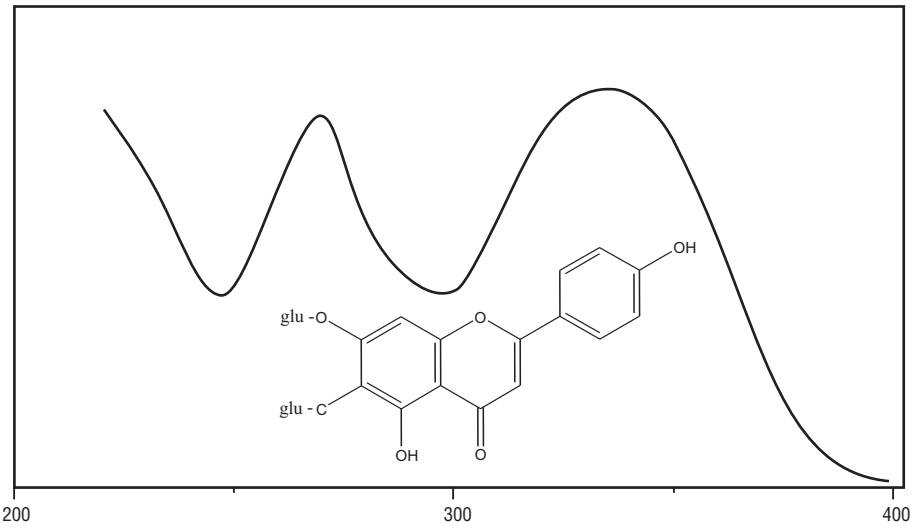
34 – 2''-*O*-glucosylvitexin

(RT = 31.7; $\lambda_{max.}$ = 268, 294sh, 336 nm)



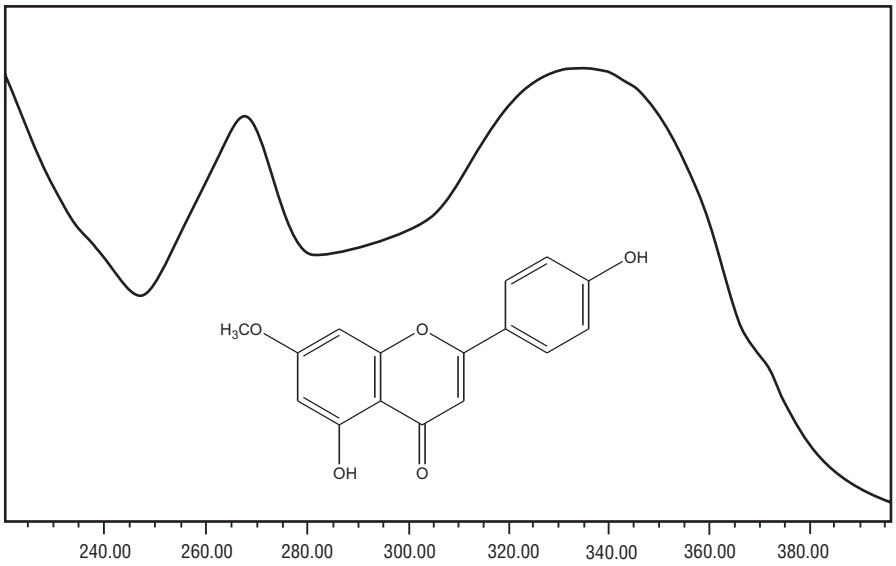
35 – saponarin

(RT = 26.1; $\lambda_{max.}$ = 268, 336 nm)



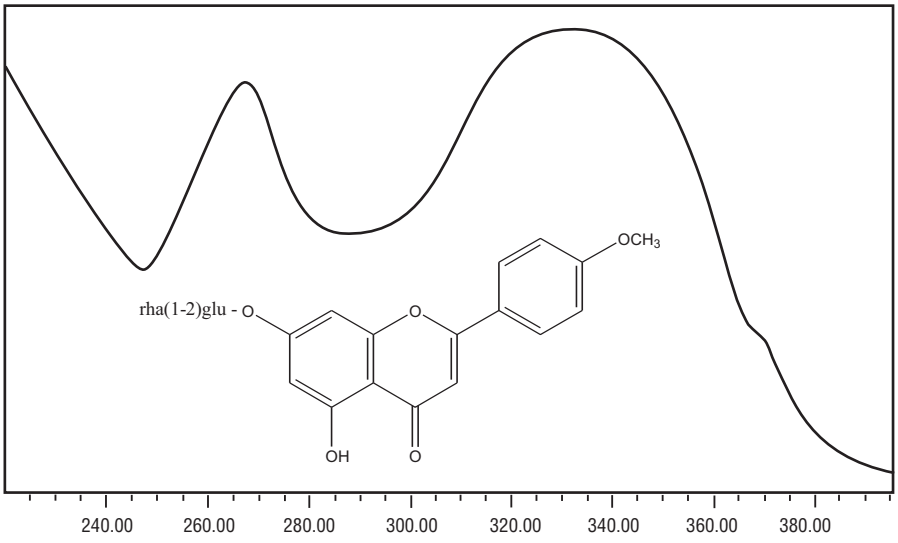
36 – genkwanin

(RT = 57.1; $\lambda_{max.}$ = 267, 290sh, 331 nm)



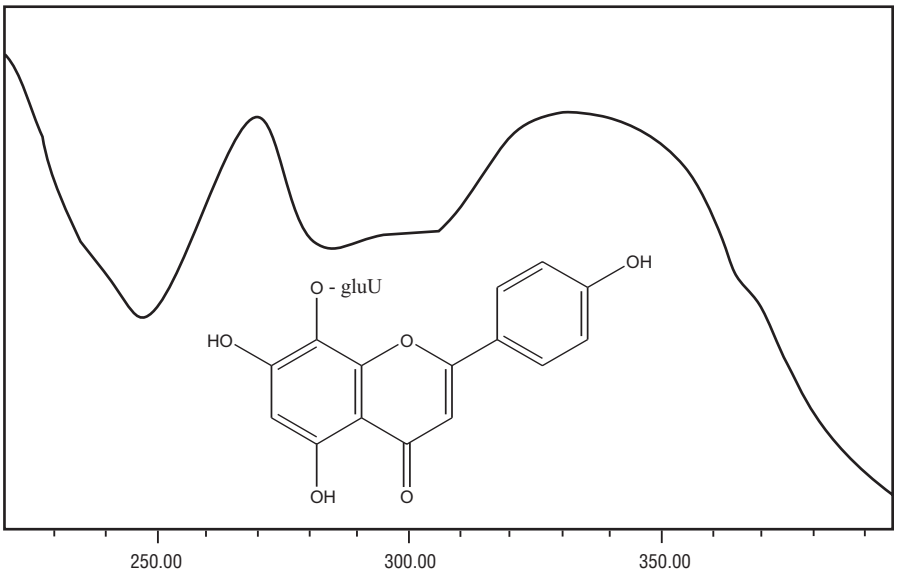
37 – acacetin-7-*O*-[rhamnosyl(1-2)glucoside]

(RT = 41.4; $\lambda_{max.}$ = 267, 332 nm)



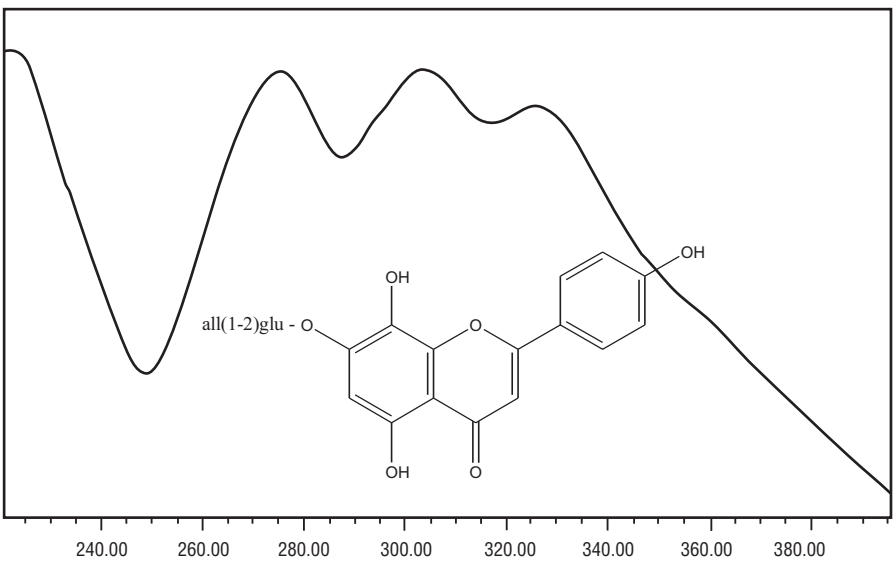
38 – 8-hydroxy-apigenin-8-*O*-glucuronide

(RT = 39.5; $\lambda_{max.}$ = 238sh, 270, 295sh, 330 nm)



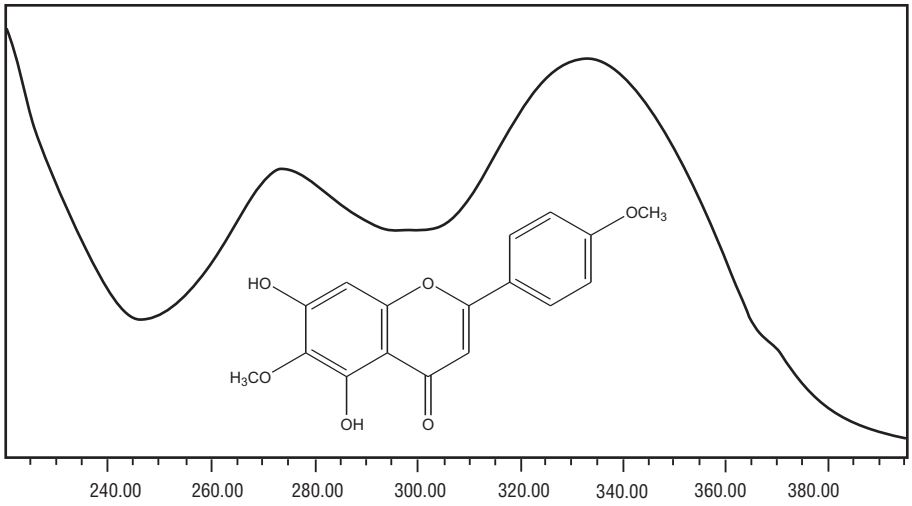
39 – 8-hydroxy-apigenin-7-*O*-[allosyl(1-2)glucoside]

(RT = 35.2; $\lambda_{max.}$ = 274, 303, 326 nm)



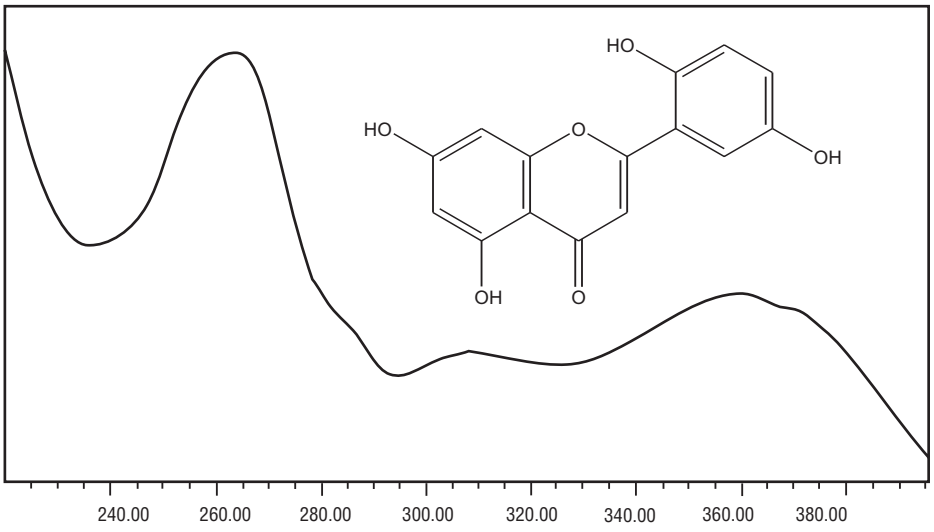
40 – **scutellarein-6,4'-dimethylether**

(RT = 58 ; $\lambda_{max.}$ = 274, 332 nm)



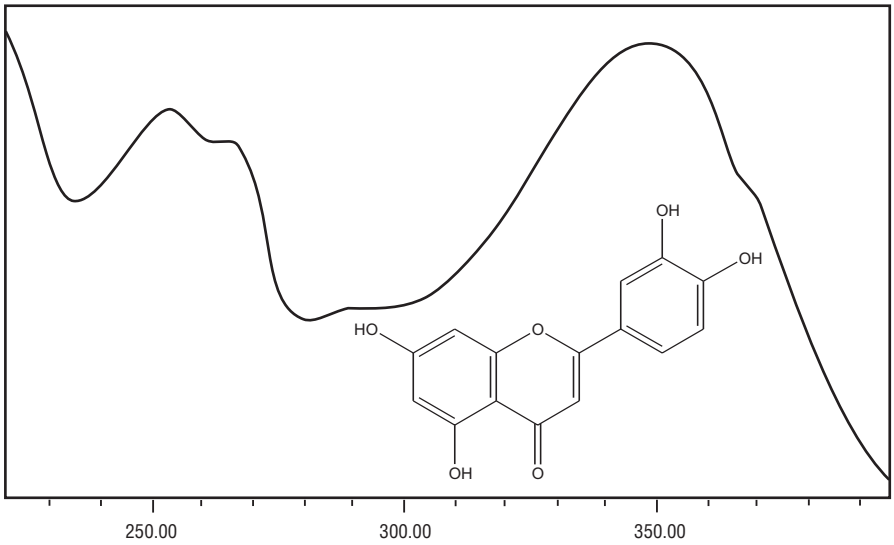
41 – 5,7,2',5'-**tetrahydroxyflavone**

(RT = 40.2; $\lambda_{max.}$ = 256sh, 263, 286sh, 308, 359 nm)



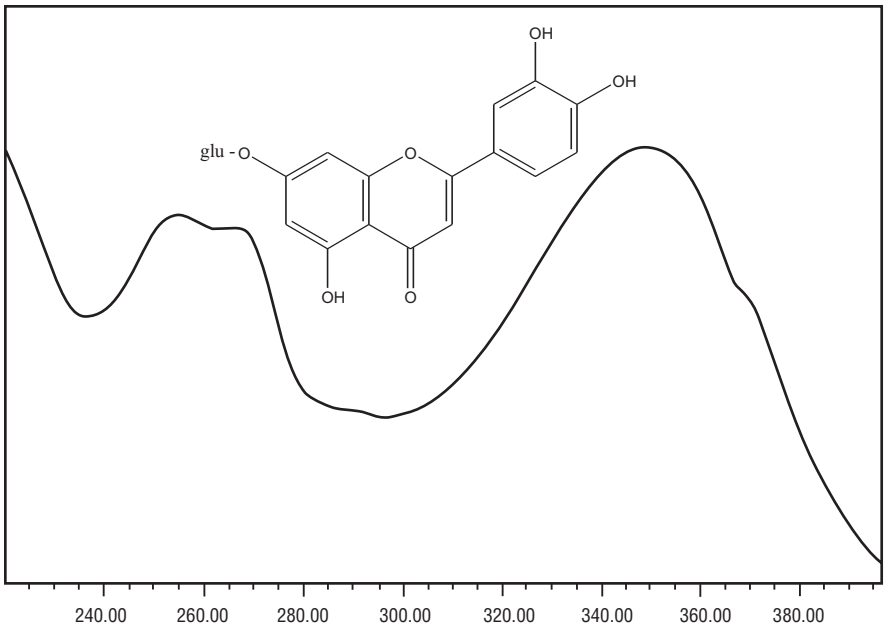
42 – luteolin

(RT = 41.6; $\lambda_{max.}$ = 253, 267sh, 291sh, 350 nm)



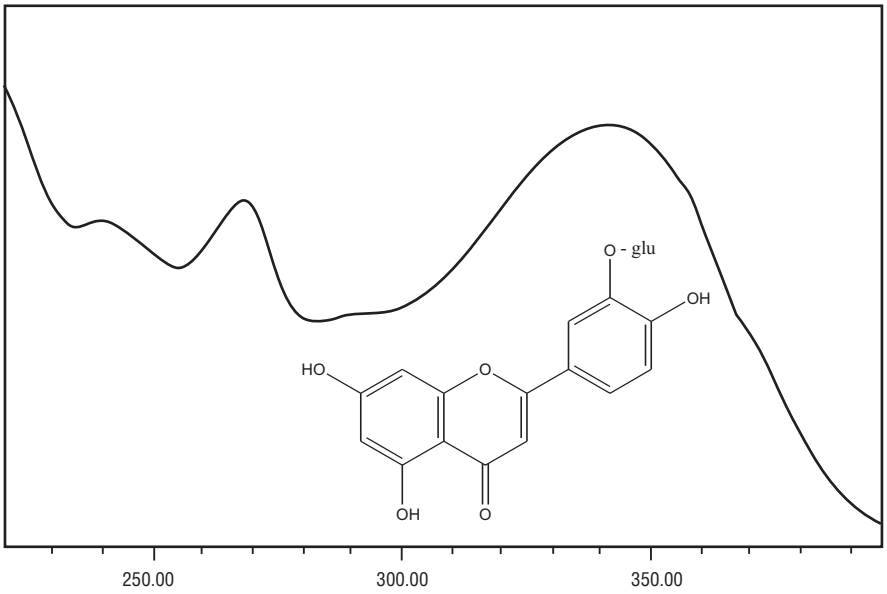
43 – luteolin-7-*O*-glucoside

(RT = 33.6; $\lambda_{max.}$ = 254, 267sh, 348 nm)



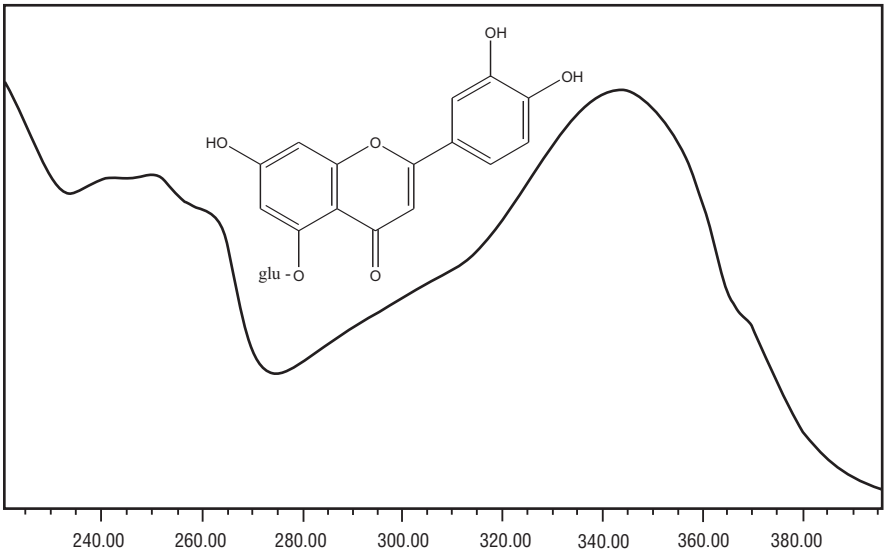
44 – luteolin-3'-*O*-glucoside

(RT = 36.5; $\lambda_{max.}$ = 240, 268, 288sh, 342 nm)



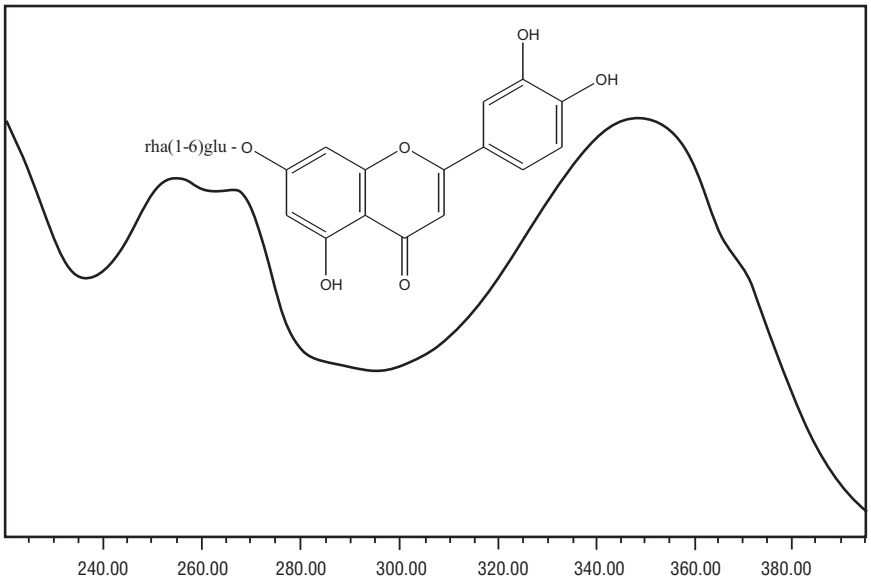
45 – luteolin-5-*O*-glucoside

(RT = 31.5; $\lambda_{max.}$ = 240sh, 249, 262sh, 288sh, 302sh, 343 nm)



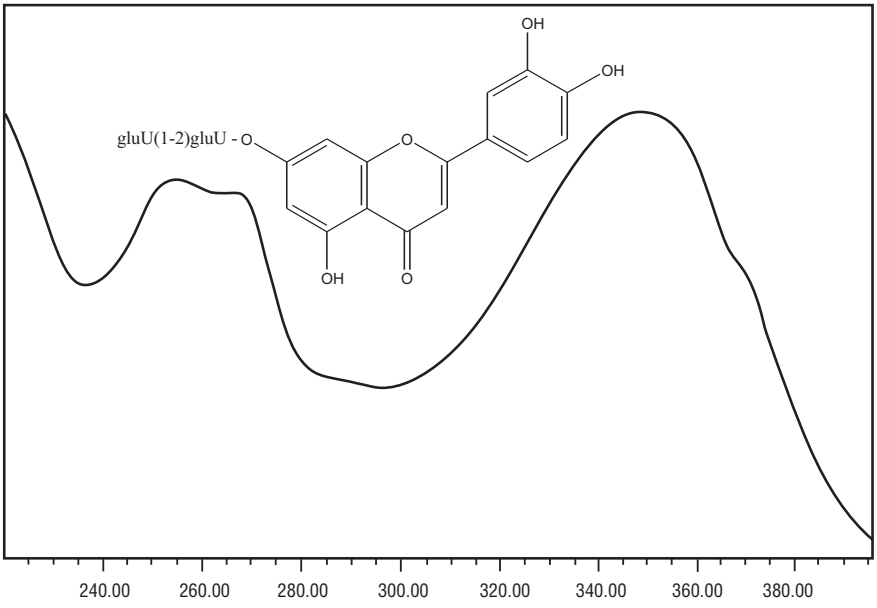
46 – luteolin-7-*O*-rutinoside

(RT = 32.7; $\lambda_{max.}$ = 254, 267sh, 348 nm)



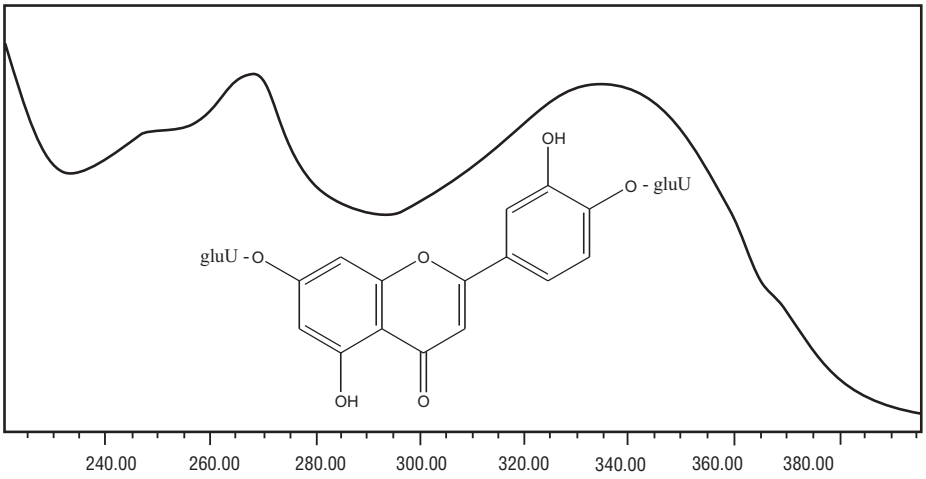
47 – luteolin-7-*O*-[glucuronosyl(1-2)glucuronide]

(RT = 29.8; $\lambda_{max.}$ = 255, 267sh, 345 nm)



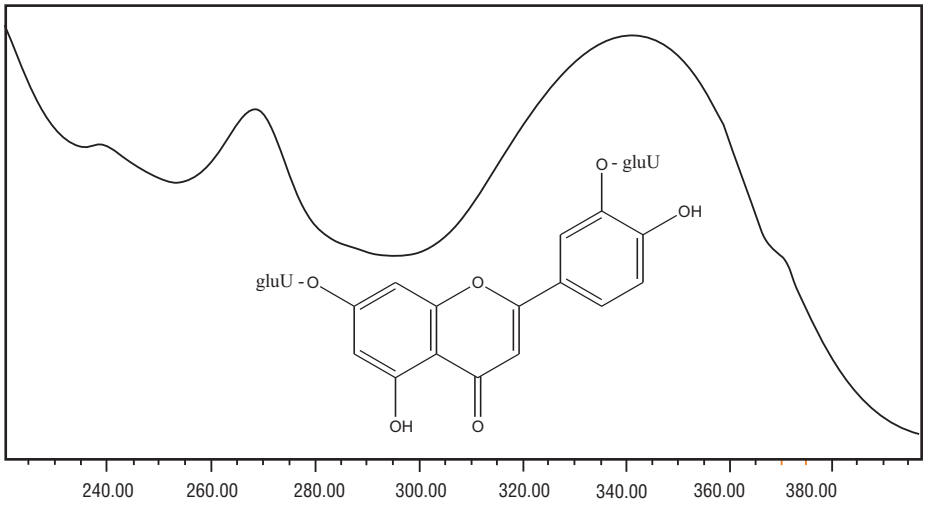
48 – luteolin-7,4'-di-*O*-glucuronide

(RT = 30.7; $\lambda_{max.}$ = 247sh, 267, 335 nm)



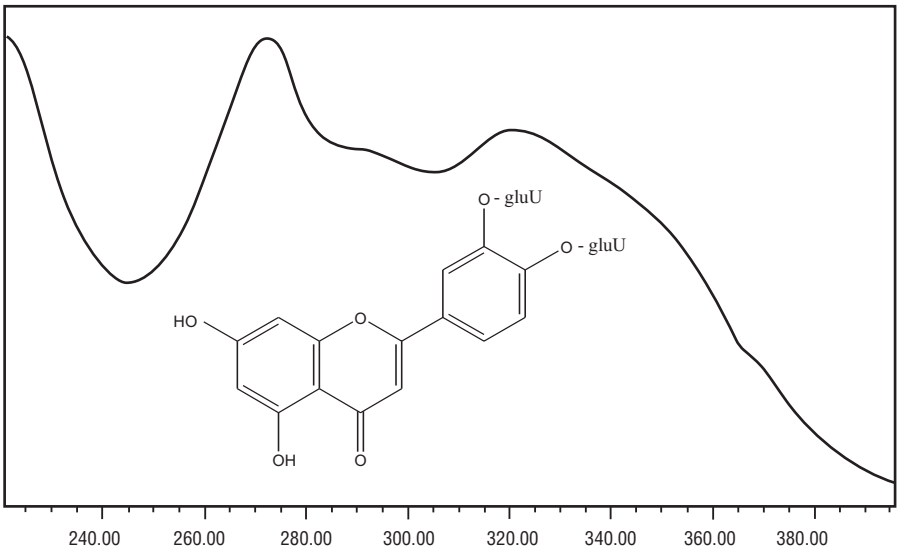
49 – luteolin-7,3'-di-*O*-glucuronide

(RT = 33.1; $\lambda_{max.}$ = 240, 268, 340 nm)



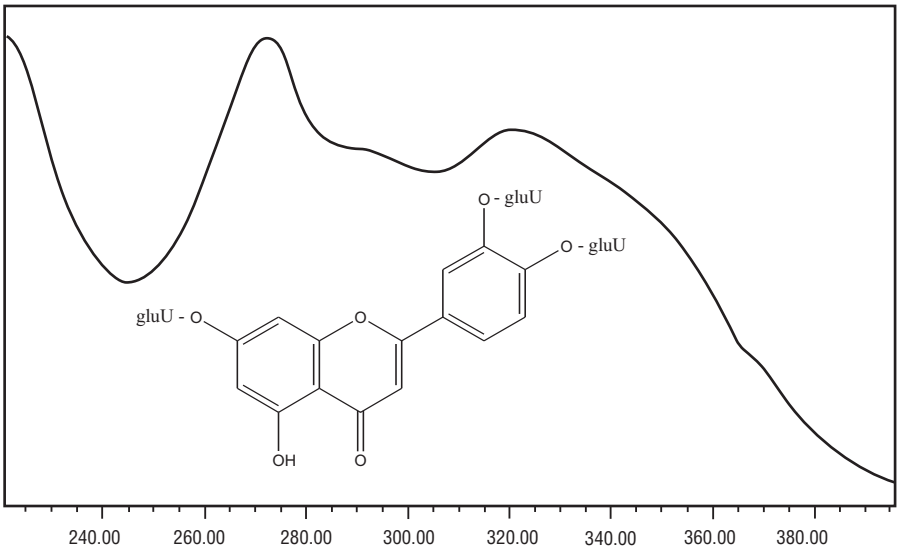
50 – luteolin-3',4'-di-*O*-glucuronide

(RT = 34.5; $\lambda_{max.}$ = 271, 291sh, 323 nm)



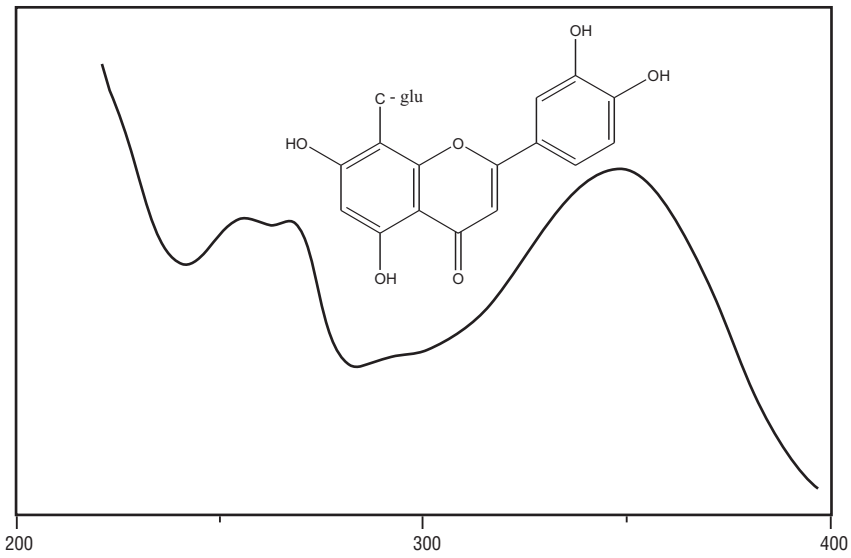
51 – luteolin-7,3',4'-tri-*O*-glucuronide

(RT = 28; $\lambda_{max.}$ = 272, 293sh, 321 nm)



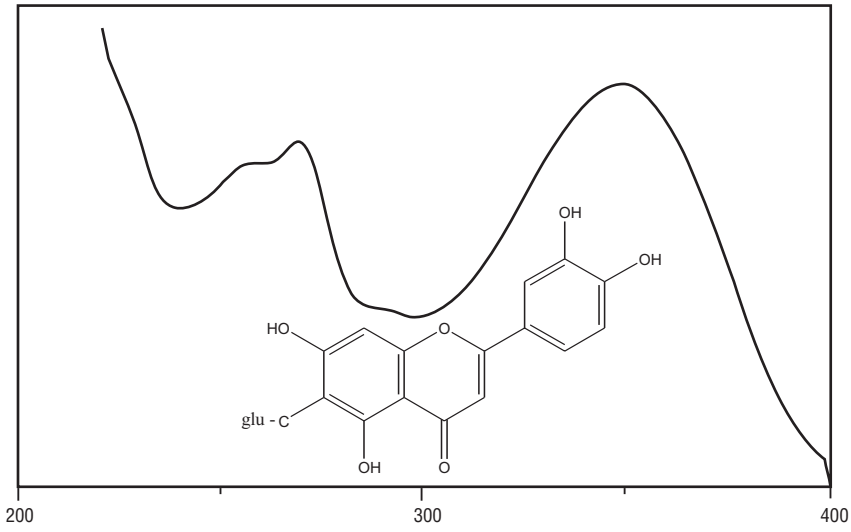
52 – orientin

(RT = 27.1; $\lambda_{max.}$ = 254, 268sh, 290sh, 350 nm)



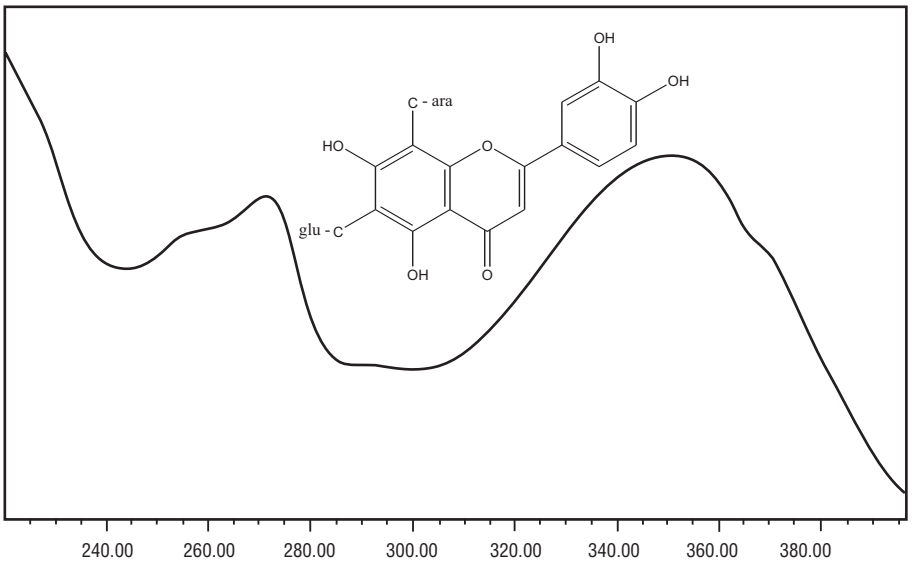
53 – isoorientin

(RT = 27.0; $\lambda_{max.}$ = 254sh, 268, 290sh, 350 nm)



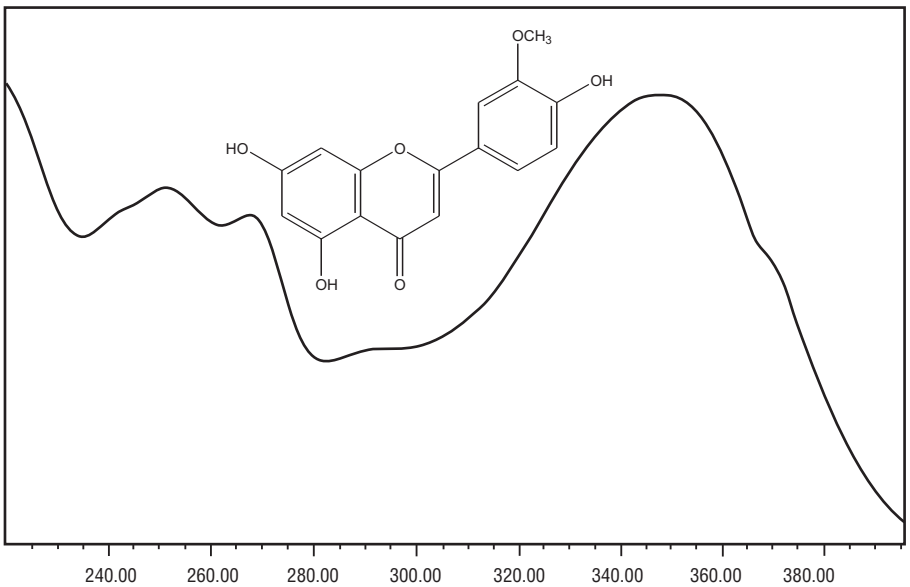
54 – neocarlinoside

(RT = 28.9; $\lambda_{max.}$ = 256sh, 271, 294sh, 350 nm)



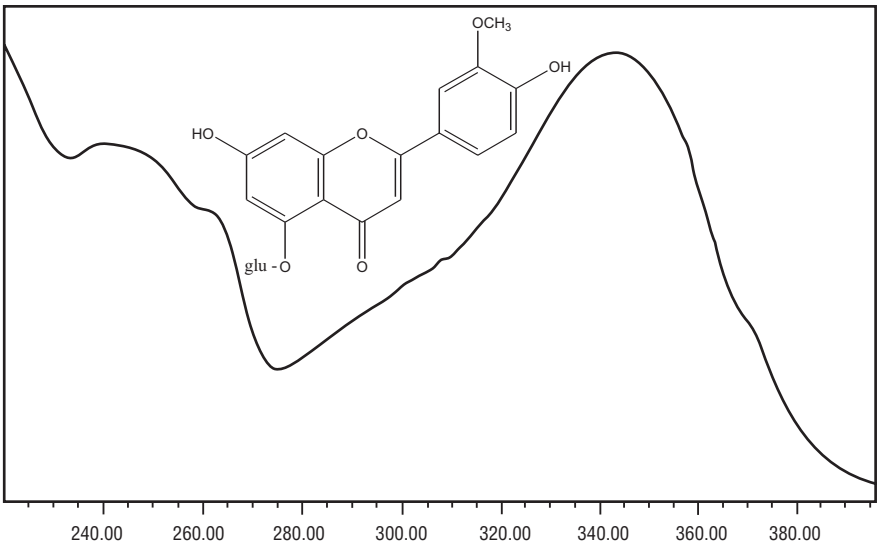
55 – chrysoeriol

(RT = 46.7; $\lambda_{max.}$ = 241sh, 252, 269, 290sh, 348 nm)



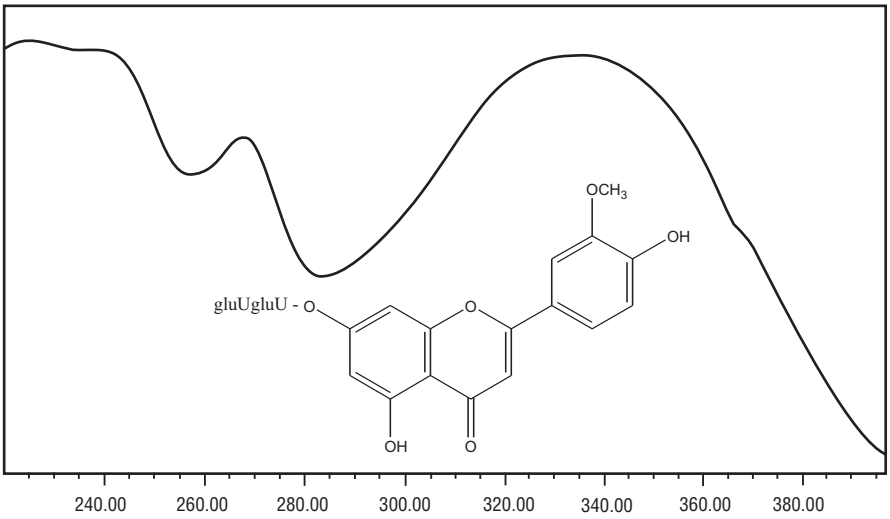
56 – chrysoeriol-5-*O*-glucoside

(RT = 35; $\lambda_{max.}$ = 238sh, 248sh, 262sh, 343 nm)



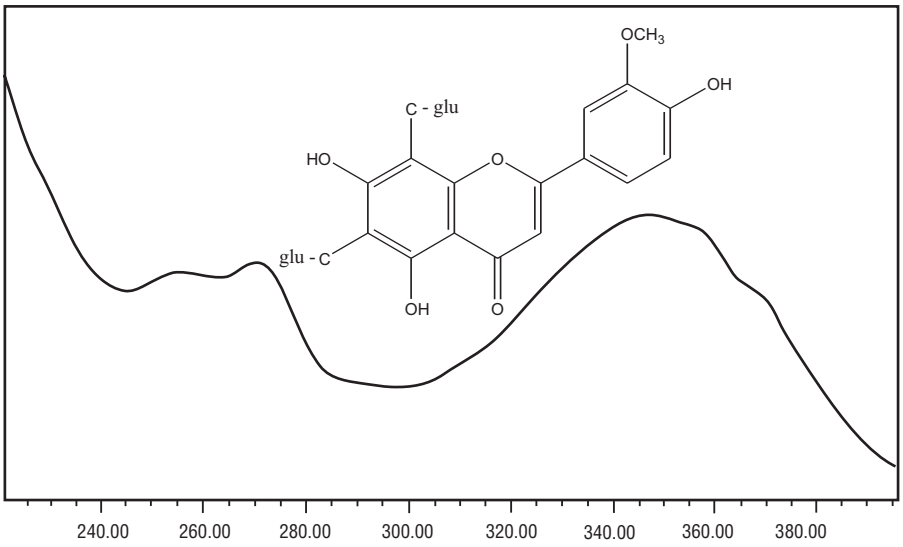
57 – chrysoeriol-7-*O*-glucuro-glucuronide

(RT = 26.8; $\lambda_{max.}$ = 225, 242sh, 267, 333 nm)



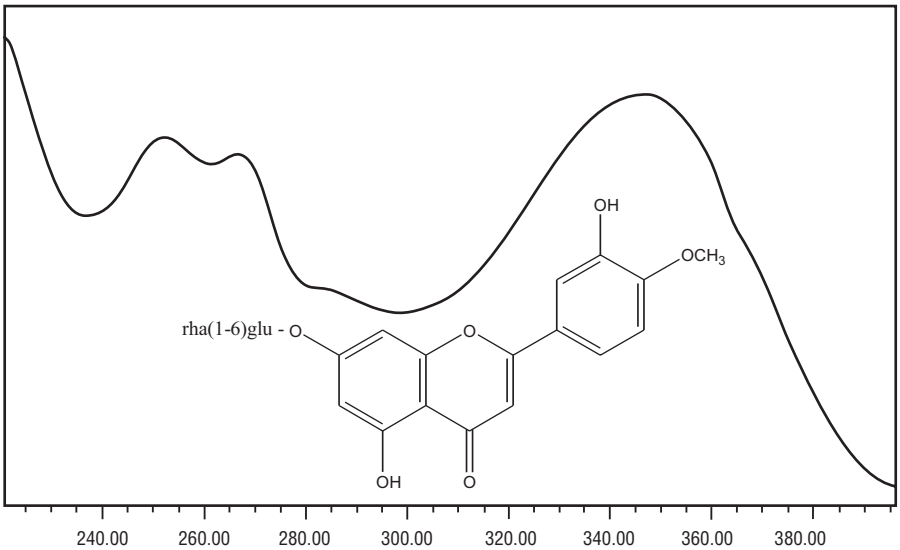
58 – **chrysoeriol-6,8-di-*C*-glucoside**

(RT = 28.7; $\lambda_{max.}$ = 253sh, 267, 344 nm)



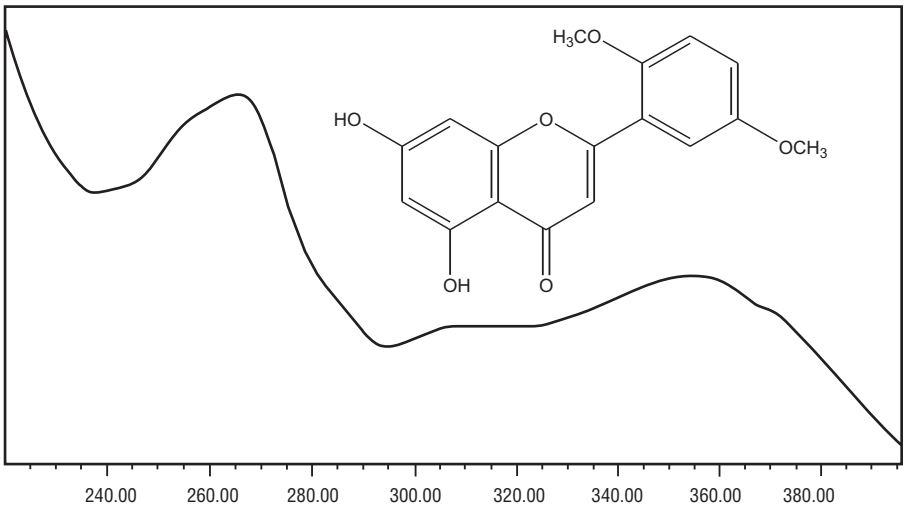
59 – **diosmetin-7-*O*-[rhamnosyl(1-6)glucoside]**

(RT = 36.3; $\lambda_{max.}$ = 251, 266sh, 346 nm)



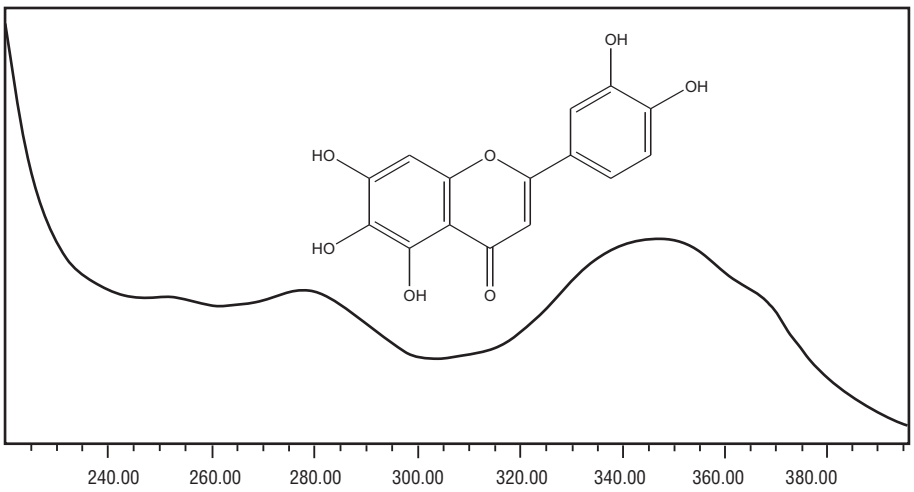
60 – 5,7-dihydroxy-2',5'-dimethoxyflavone

(RT = 58.4; $\lambda_{max.}$ = 256sh, 265, 310sh, 355 nm)

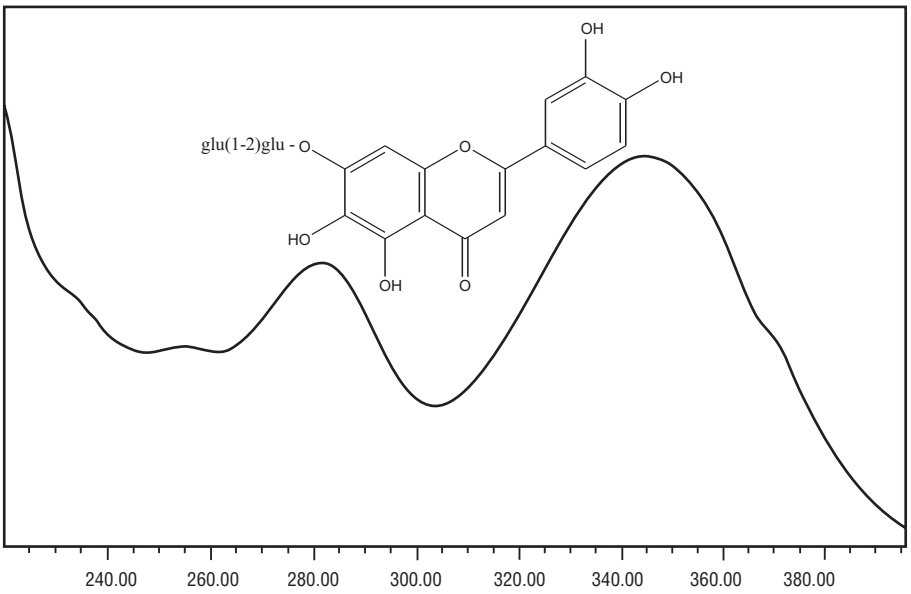


61 – 6-hydroxyluteolin

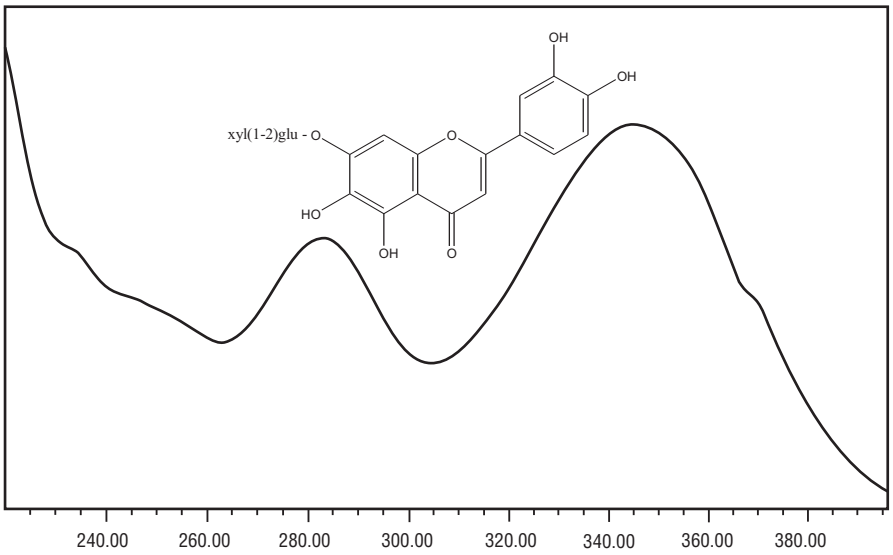
(RT = 35.7; $\lambda_{max.}$ = 251sh, 278, 310sh, 344 nm)



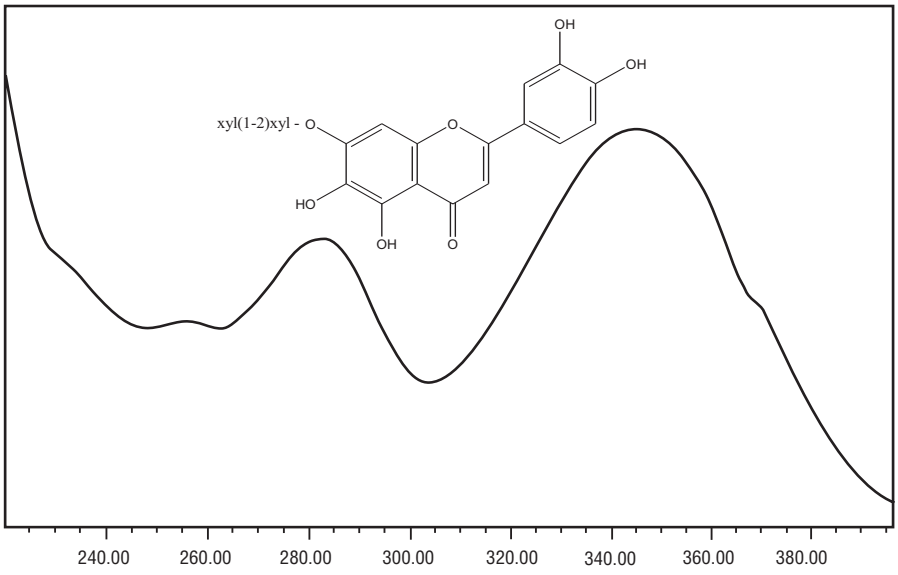
62 – 6-hydroxyluteolin-7-*O*-[glucosyl(1-2)glucoside]
(RT = 29.7; $\lambda_{max.}$ = 232sh, 253sh, 281, 344 nm)



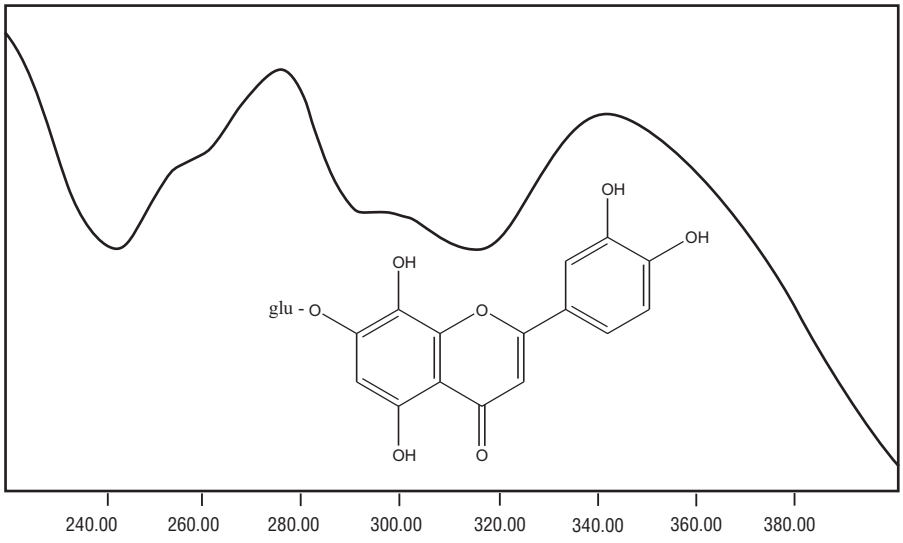
63 – 6-hydroxyluteolin-7-*O*-[xylosyl(1-2)glucoside]
(RT = 38.7; $\lambda_{max.}$ = 232sh, 245sh, 253sh, 282, 345 nm)



64 – 6-hydroxyluteolin-7-*O*-[xylosyl(1-2)xyloside]
(RT = 34.9; $\lambda_{max.}$ = 232sh, 253sh, 282, 344 nm)

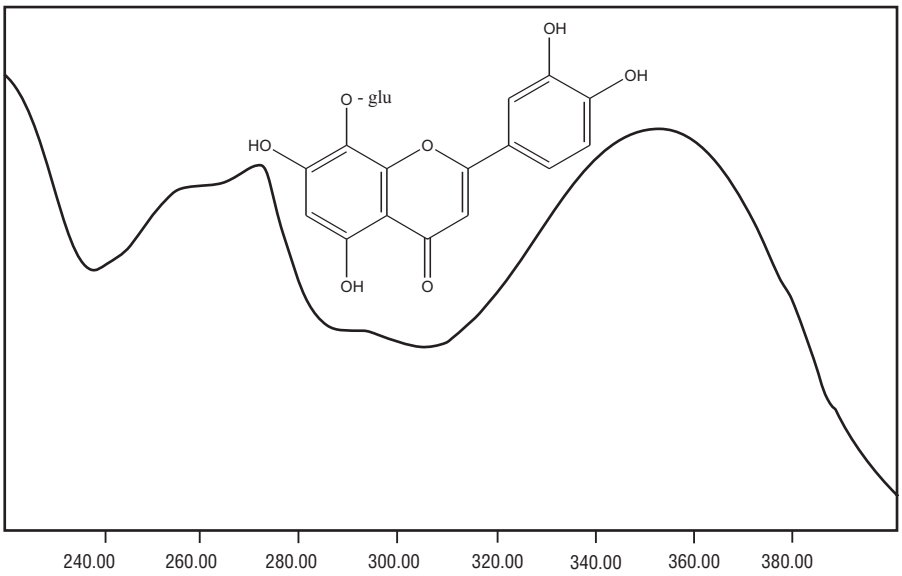


65 – 8-hydroxyluteolin-7-*O*-glucoside
(RT = 29.6; $\lambda_{max.}$ = 255sh, 277, 298sh, 340 nm)



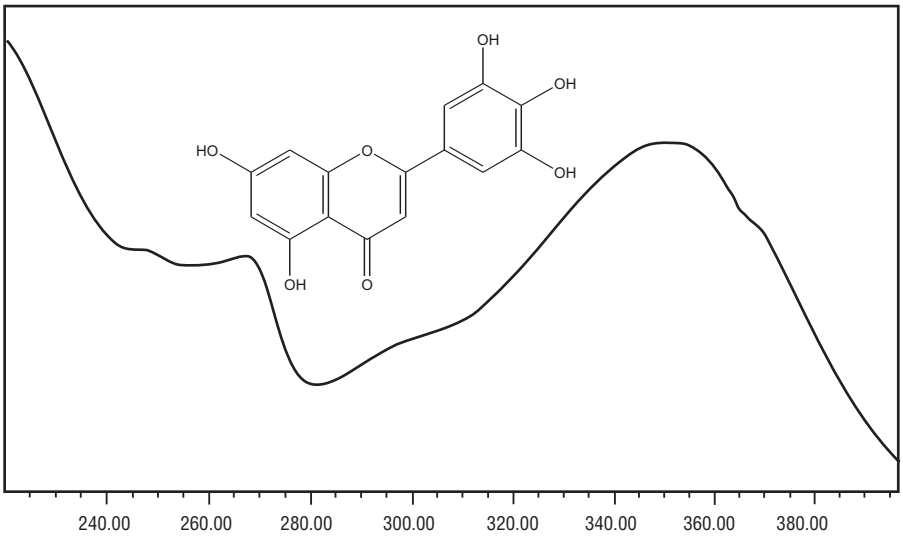
66 – 8-hydroxy-luteolin-8-*O*-glucoside

(RT = 35.1; $\lambda_{max.}$ = 255sh, 270, 292sh, 352 nm)



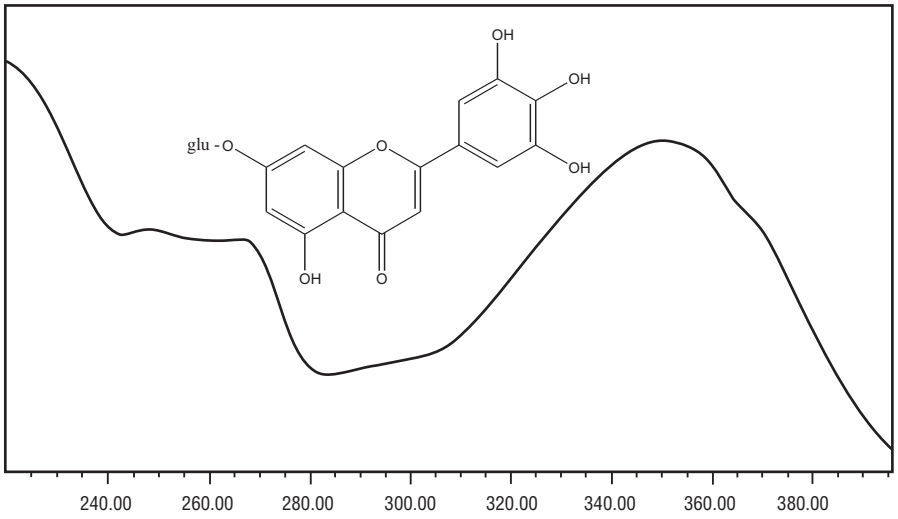
67 – tricetin

(RT = 38.0; $\lambda_{max.}$ = 247sh, 266, 296sh, 350 nm)



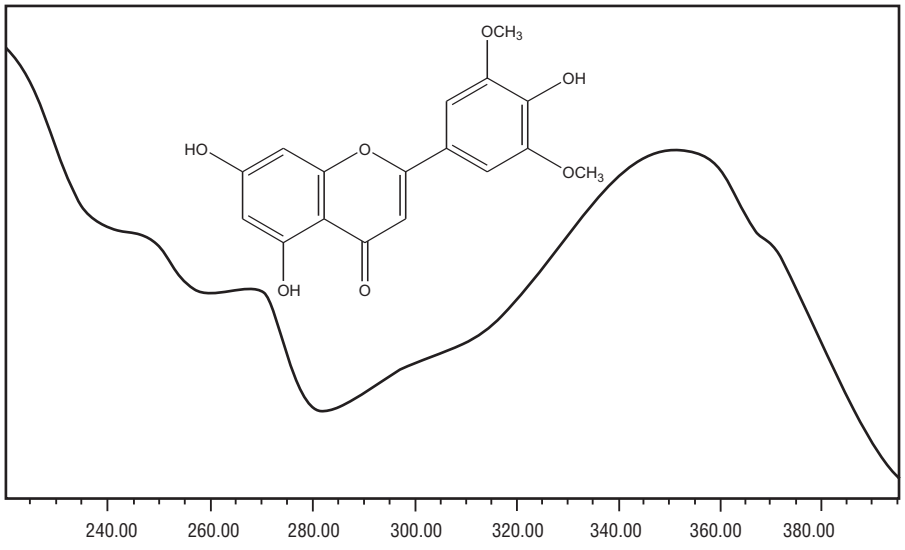
68 – tricetin-7-*O*-glucoside

(RT = 30.7; $\lambda_{max.}$ = 247sh, 266, 350 nm)



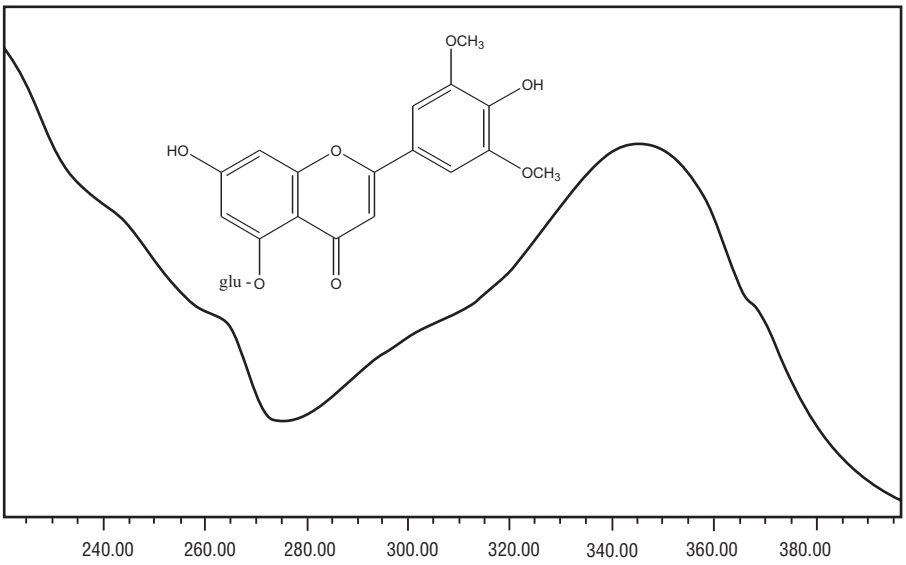
69 – tricin

(RT = 46.1; $\lambda_{max.}$ = 245, 266, 296sh, 354 nm)



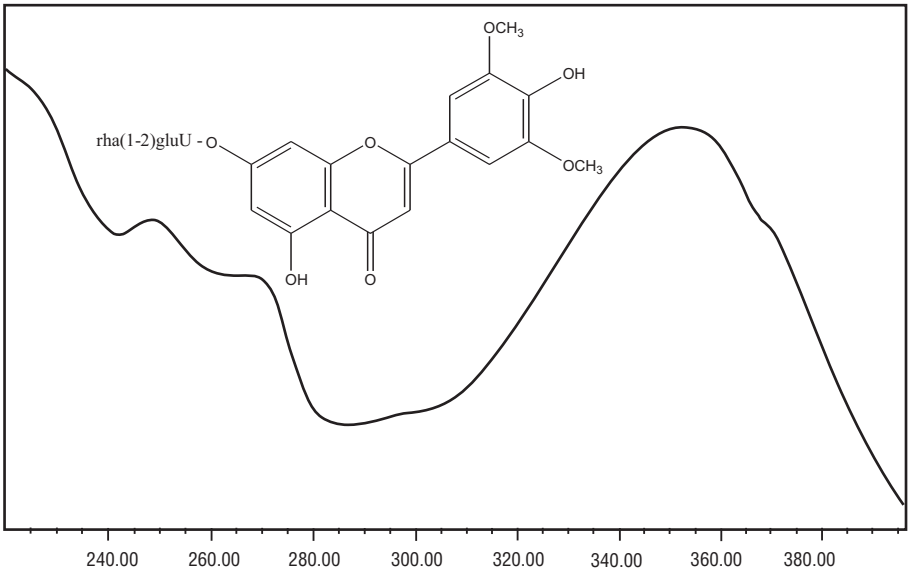
70 – tricin-5-*O*-glucoside

(RT = 35; λmax. = 245, 266, 296sh, 351 nm)



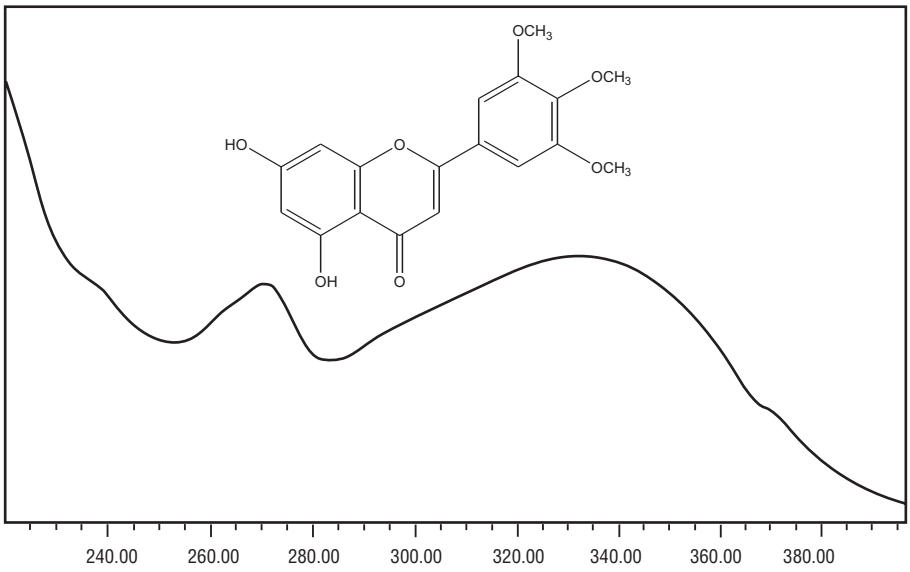
71 – tricin-7-*O*-[rhamnosyl(1-2)glucuronide]

(RT = 36.2; $\lambda_{max.}$ = 248, 267, 296sh, 351 nm)



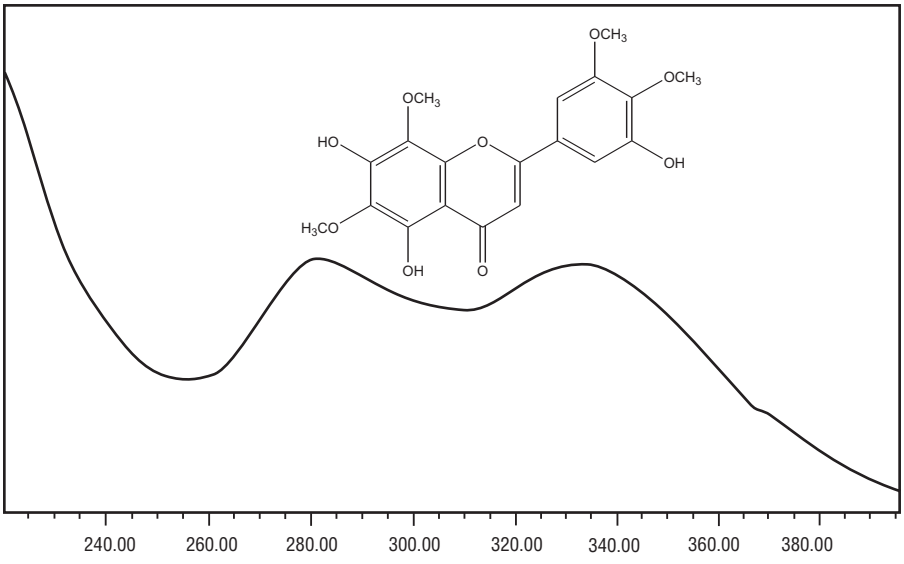
72 – **tricetin-3',4',5'-trimethylether**

(RT = 54.7; $\lambda_{max.}$ = 237sh, 269, 331 nm)

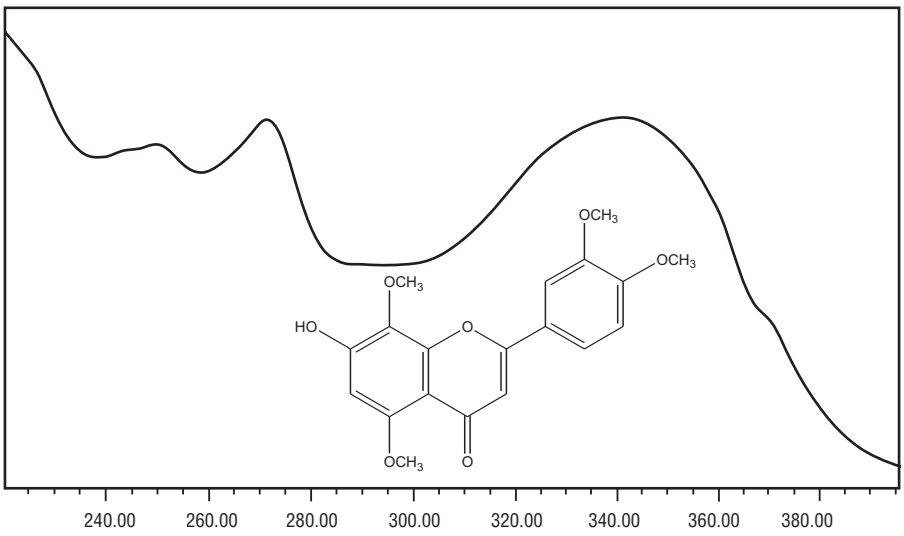


73 – **scaposin**

(RT = 48.3; $\lambda_{max.}$ = 280, 331 nm)



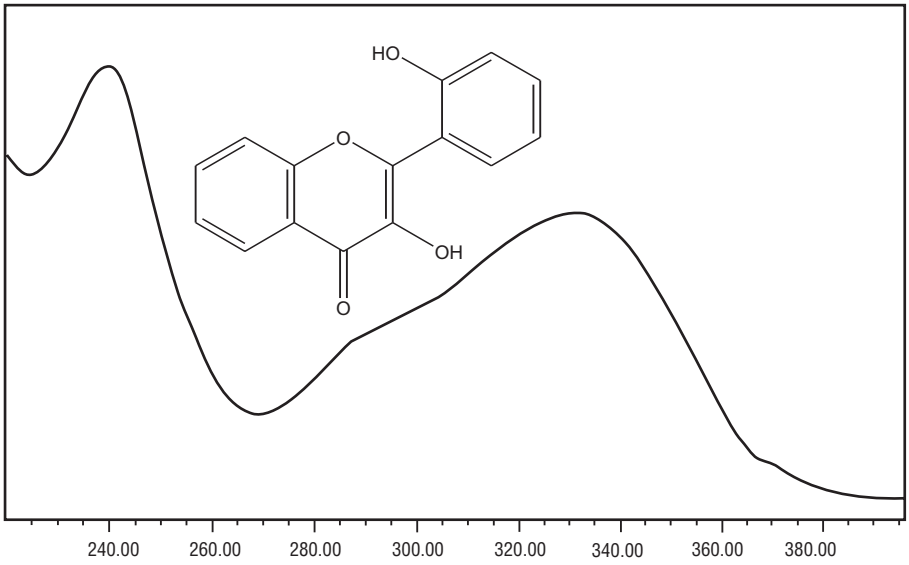
74 – 7-hydroxy-5,8,3',4'-tetramethoxyflavone
(RT = 42.6; $\lambda_{max.}$ = 249, 270, 340 nm)



4. FLAVONOLS

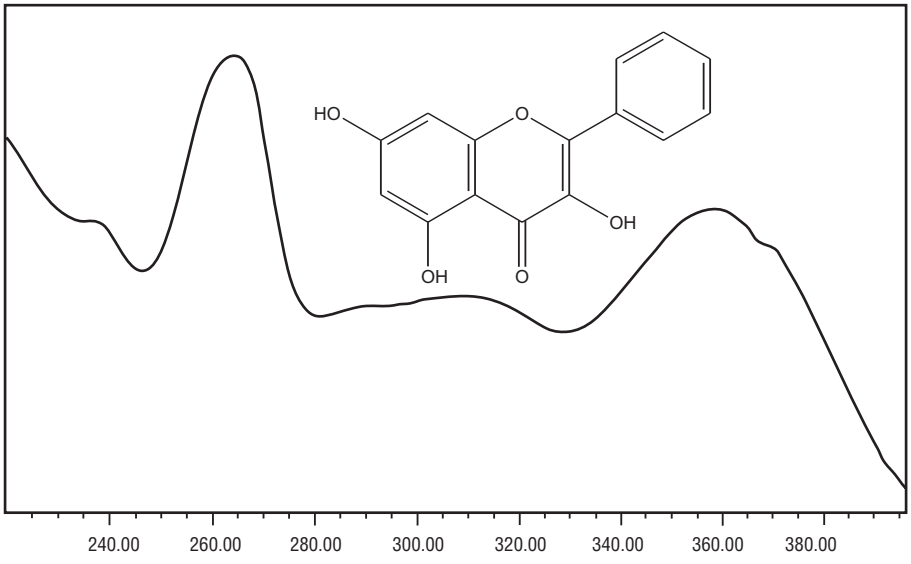
75 – 2'-hydroxyflavonol

(RT = 48.8; $\lambda_{max.}$ = 240, 292sh, 330 nm)



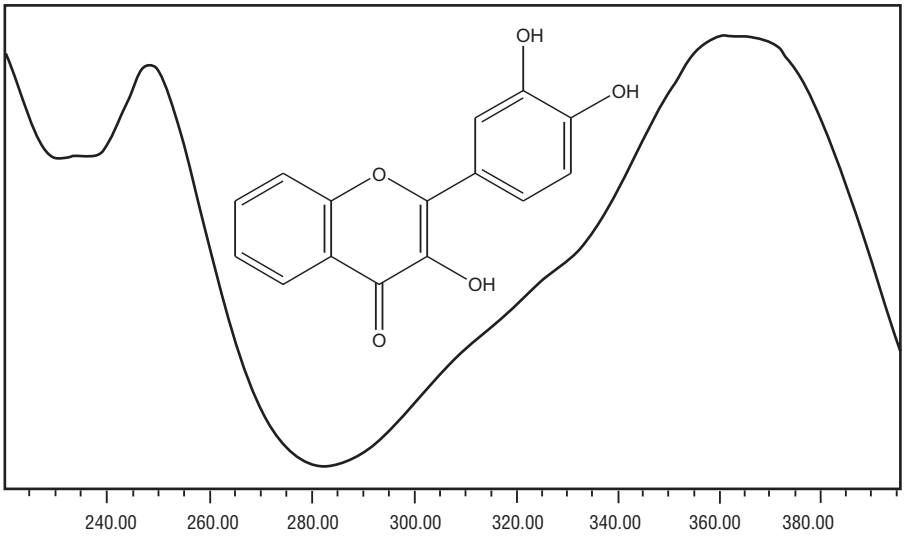
76 – galangin

(RT = 57.8; $\lambda_{max.}$ = 237sh, 263, 288sh, 310, 358 nm)



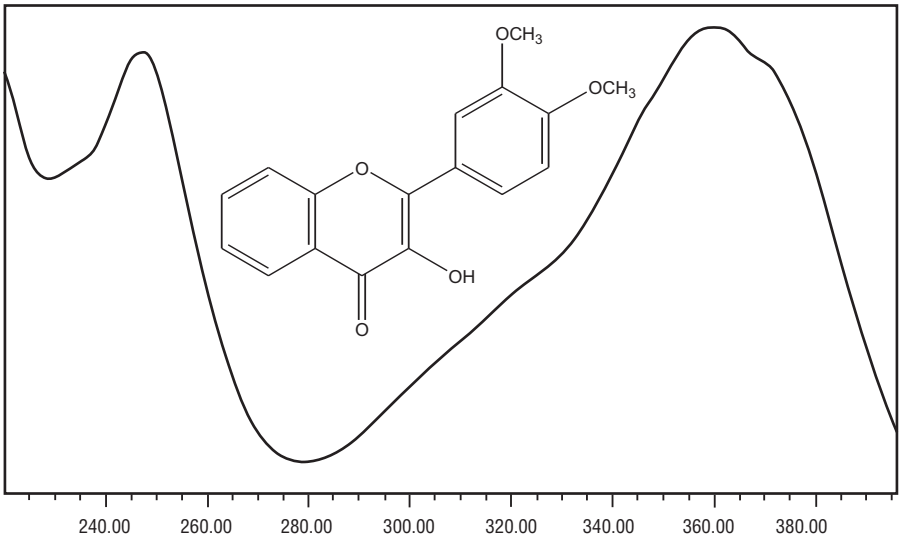
77 – 3',4'-dihydroxyflavonol

(RT = 43.7; $\lambda_{max.}$ = 230sh, 248, 312sh, 327sh, 360 nm)



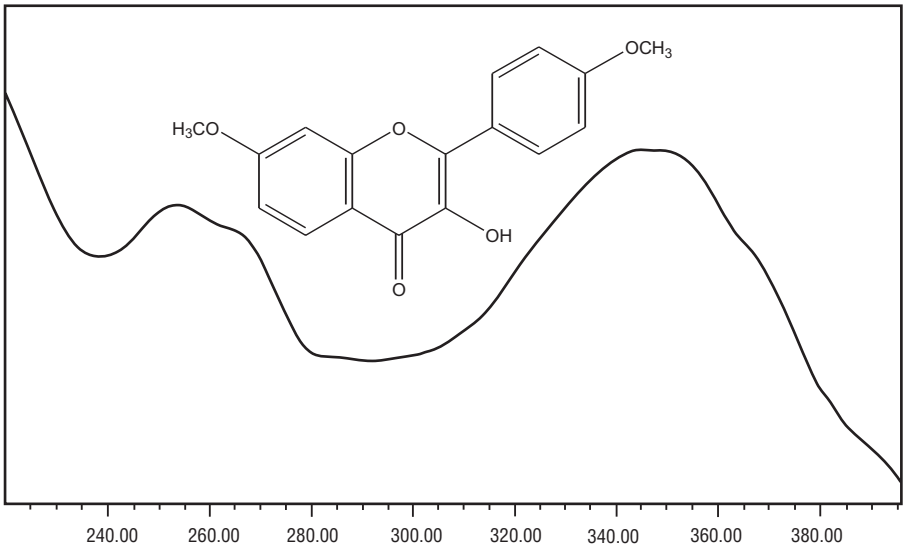
78 – 3',4'-dimethoxyflavonol

(RT = 57.2; $\lambda_{max.}$ = 230sh, 248, 312sh, 327sh, 360 nm)



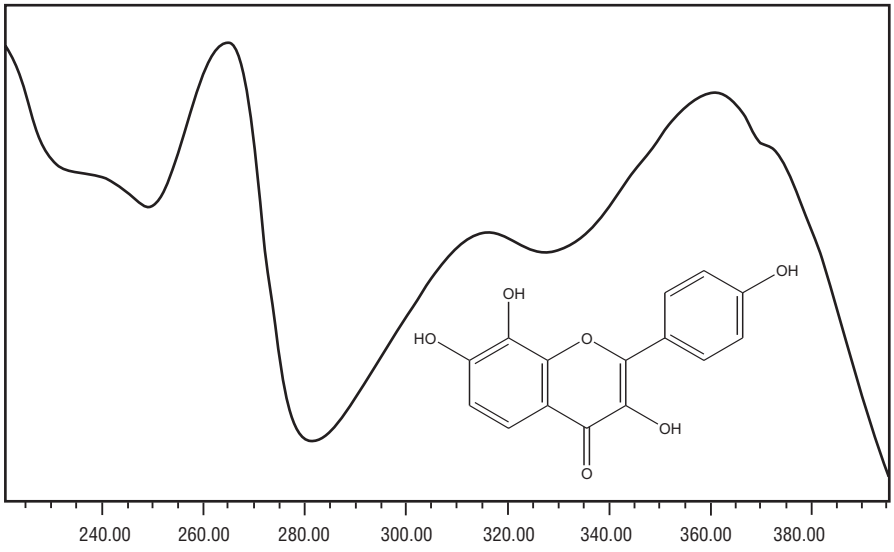
79 – 7,4'-di-*O*-dimethylflavonol

(RT = 32.8; $\lambda_{max.}$ = 254, 266sh, 346 nm)



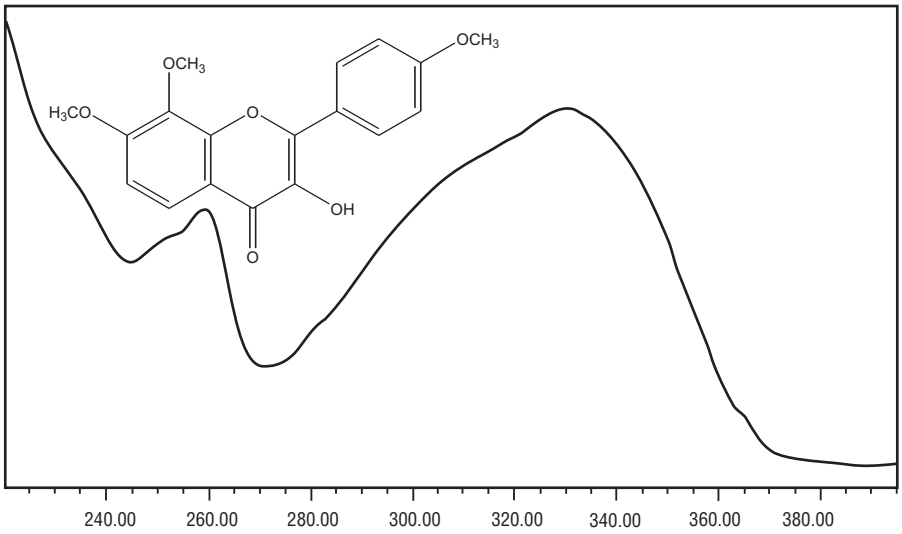
80 – 7,8,4'-trihydroxyflavonol

(RT = 36.5; $\lambda_{max.}$ = 237sh, 263, 315, 359 nm)



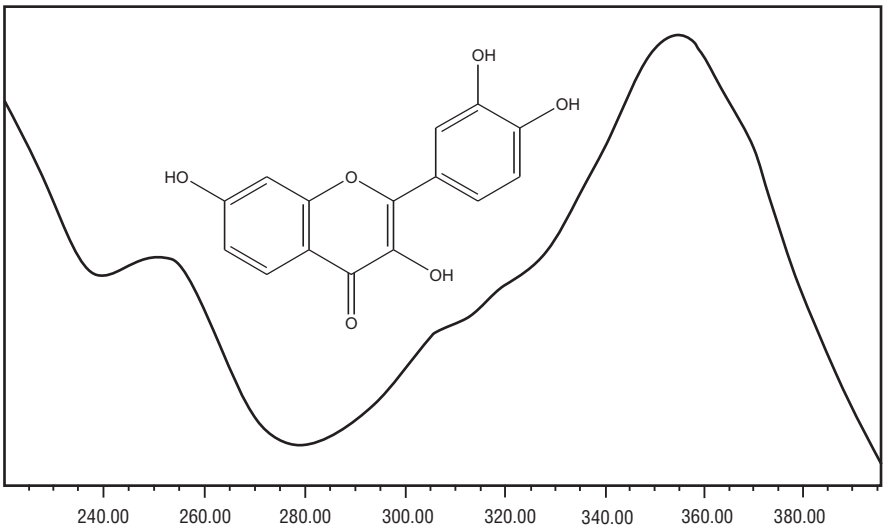
81 – 7,8,4'-trimethoxyflavonol

(RT = 58; $\lambda_{max.}$ = 250sh, 258, 308sh, 330 nm)



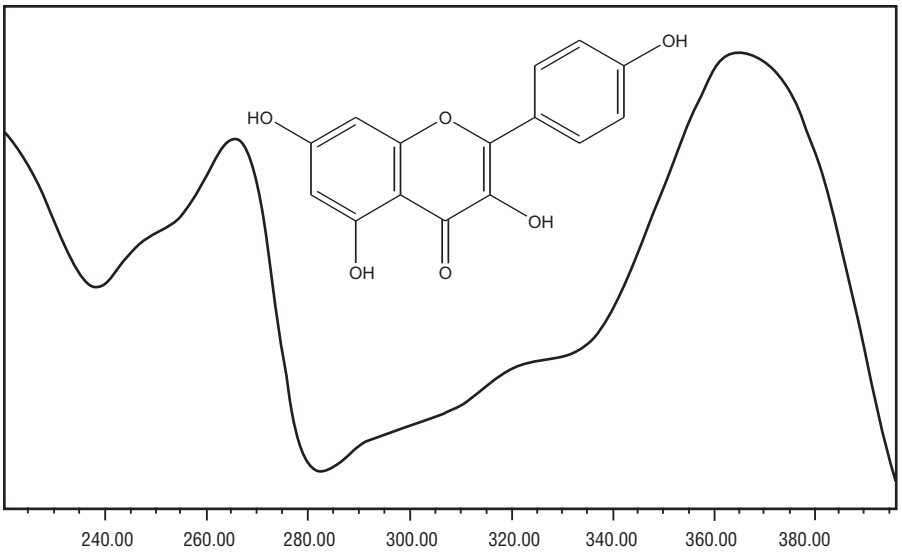
82 – fisetin

(RT = 37.2; $\lambda_{max.}$ = 253, 305, 320sh, 354 nm)



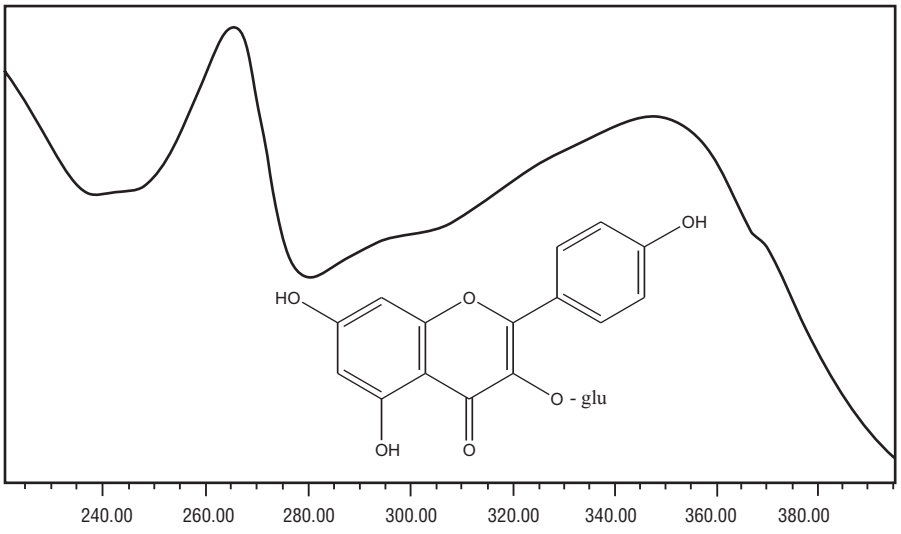
83 – **kaempferol**

(RT = 46.6; $\lambda_{max.}$ = 248sh, 266, 295sh, 318sh, 366 nm)



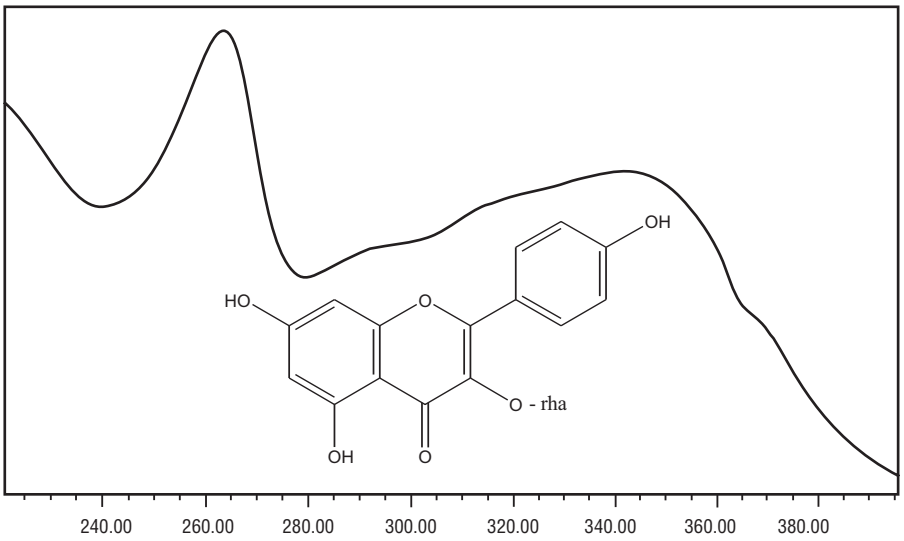
84 – **kaempferol-3-*O*-glucoside**

(RT = 35.6; $\lambda_{max.}$ = 265, 290sh, 320sh, 346 nm)



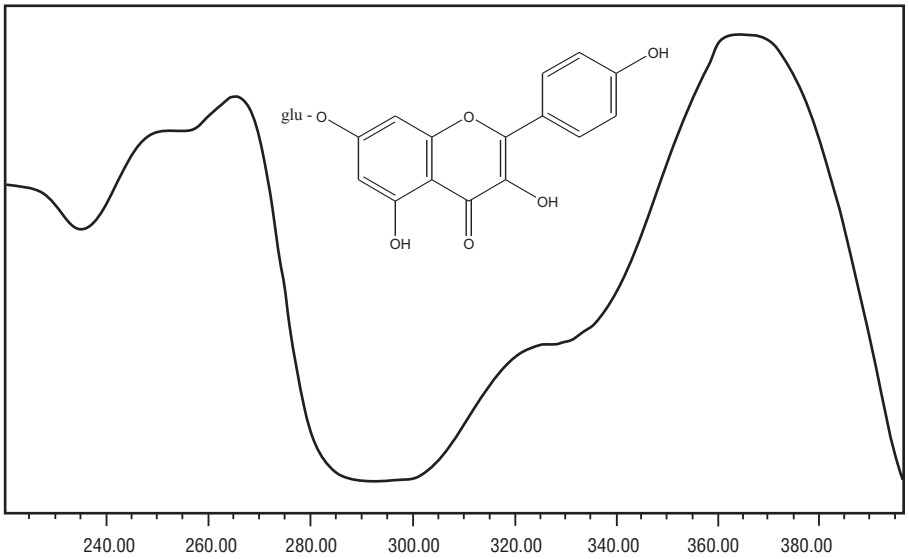
85 – **kaempferol-3-*O*-rhamnoside**

(RT=38.5; $\lambda_{max.}$= 262, 292sh, 315sh, 342 nm)

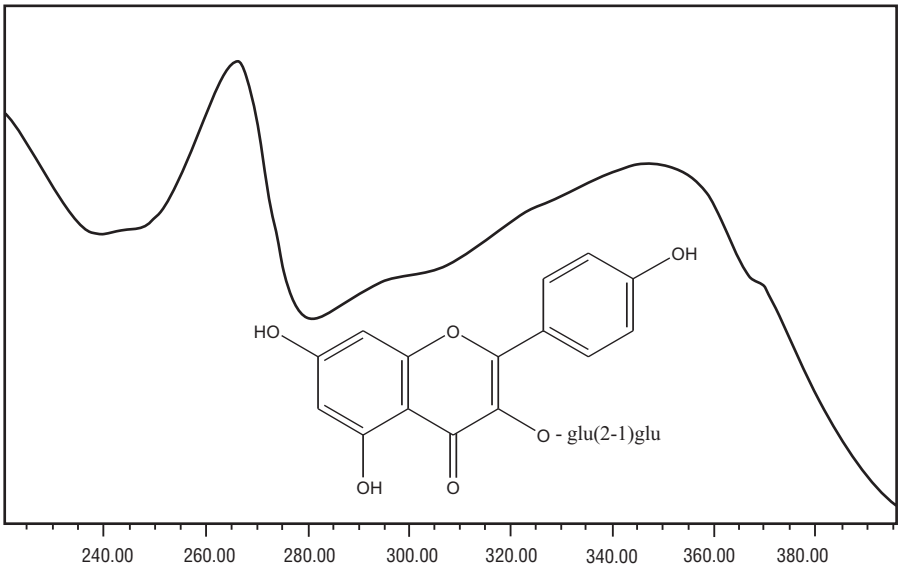


86 – **kaempferol-7-*O*-glucoside**

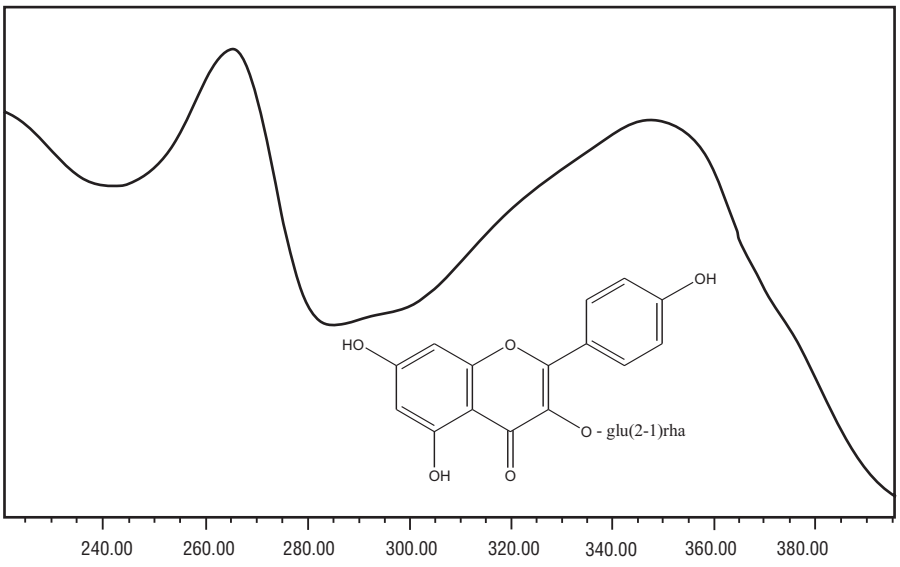
(RT = 35.8 ; $\lambda_{max.}$ = 248sh, 265, 322sh, 362 nm)



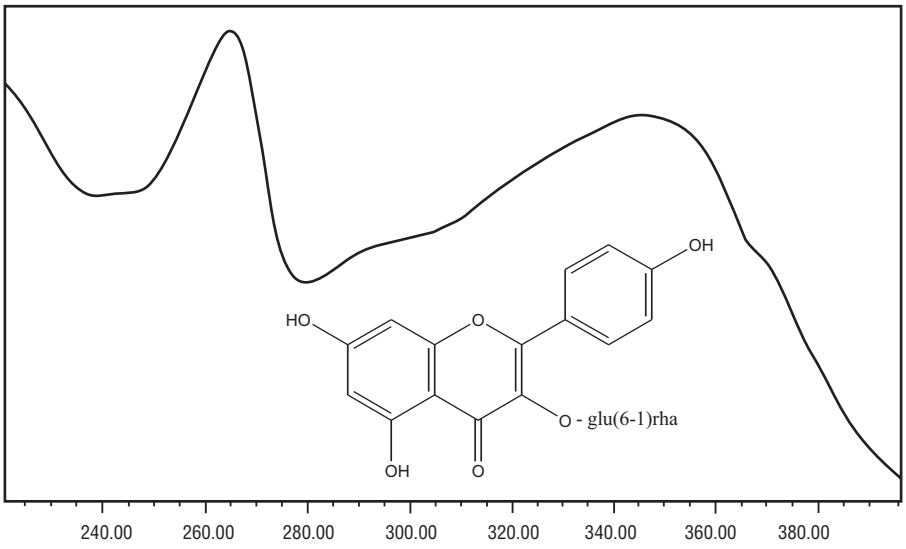
87 – kaempferol-3-*O*-[glucosyl(1-2)glucoside]
(RT= 32.1; $\lambda_{max.}$= 242sh, 265, 292sh, 322sh, 346 nm)



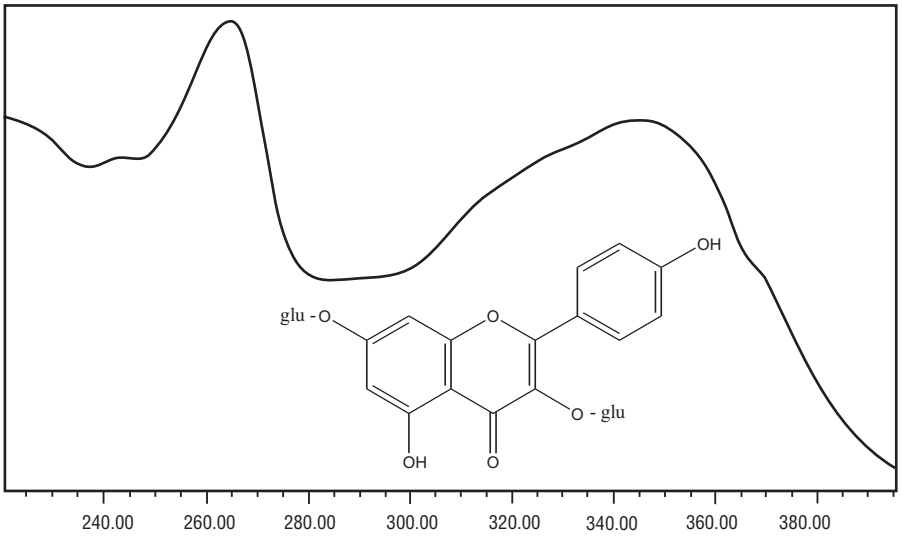
88 – kaempferol-3-*O*-[rhamnosyl(1-2)glucoside]
(RT=33.2; $\lambda_{max.}$= 265, 290sh, 320sh, 350 nm)



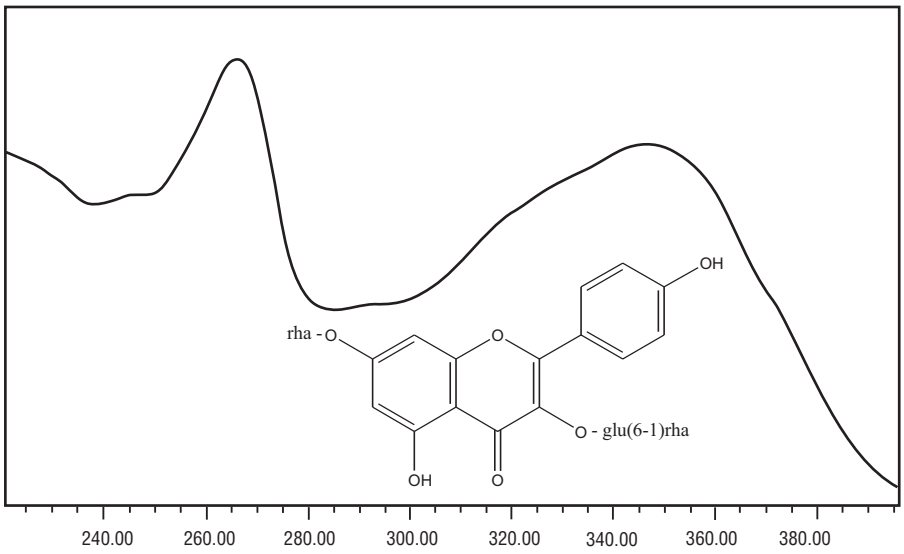
89 – kaempferol-3-*O*-[rhamnosyl(1-6)glucoside]
(RT = 35.1; $\lambda_{max.}$ = 265, 290sh, 320sh, 348 nm)



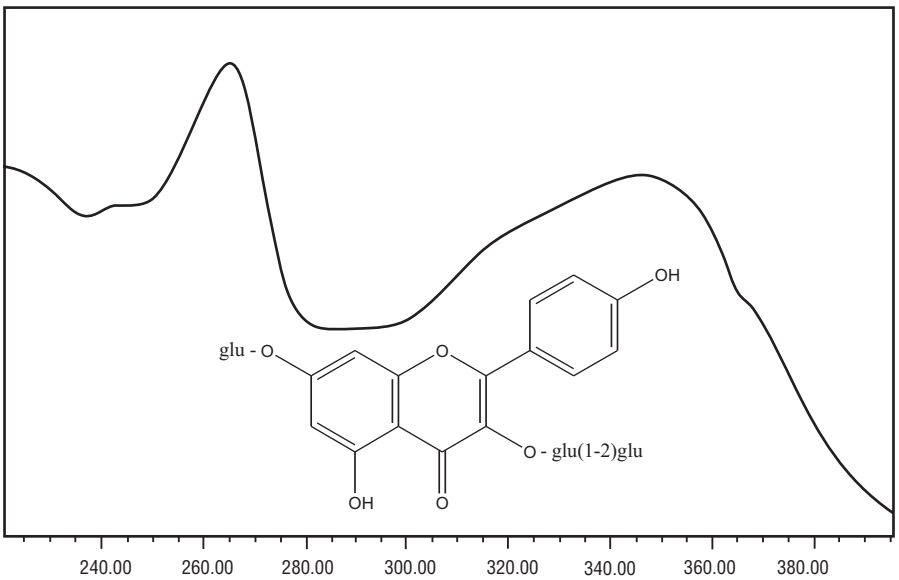
90 – kaempferol-3,7-*O*-diglucoside
(RT = 27.4; $\lambda_{max.}$ = 242sh, 265, 322sh, 345 nm)



91 – kaempferol-3-*O*-[rhamnosyl(1-6)glucoside]-7-*O*-rhamnoside
(RT = 31.8; λ_{max} = 245sh, 266, 292sh, 316sh, 345 nm)

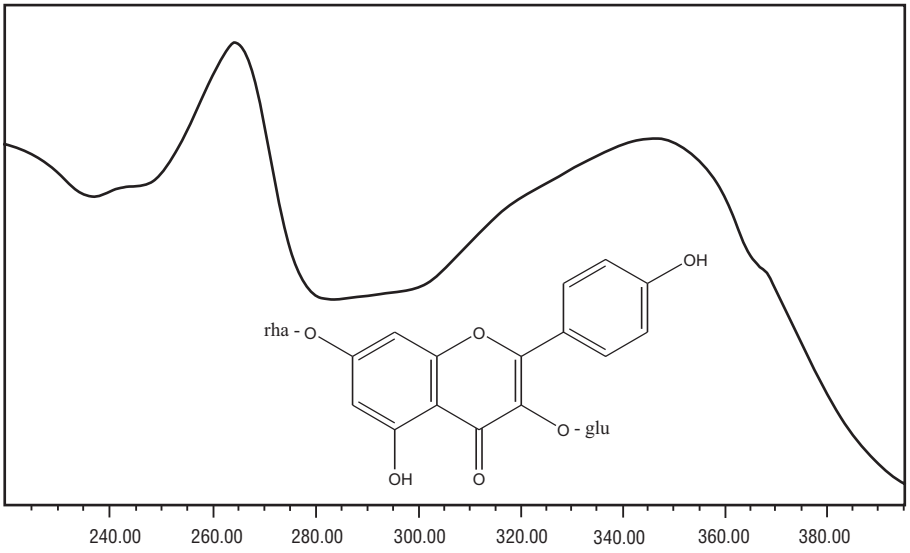


92 – kaempferol-3-*O*-[glucosyl(1-2)glucoside]-7-*O*-glucoside
(RT = 23.7; $\lambda_{max.}$ = 242sh, 263, 315sh, 344 nm)



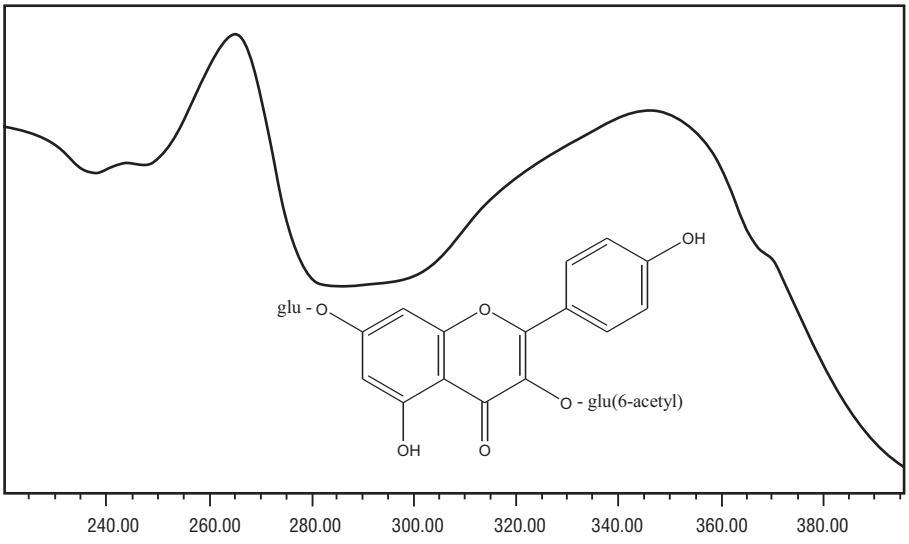
93 – kaempferol-3-*O*-glucoside-7-*O*-rhamnoside

(RT = 32; $\lambda_{max.}$ = 242sh, 265, 315sh, 344 nm)

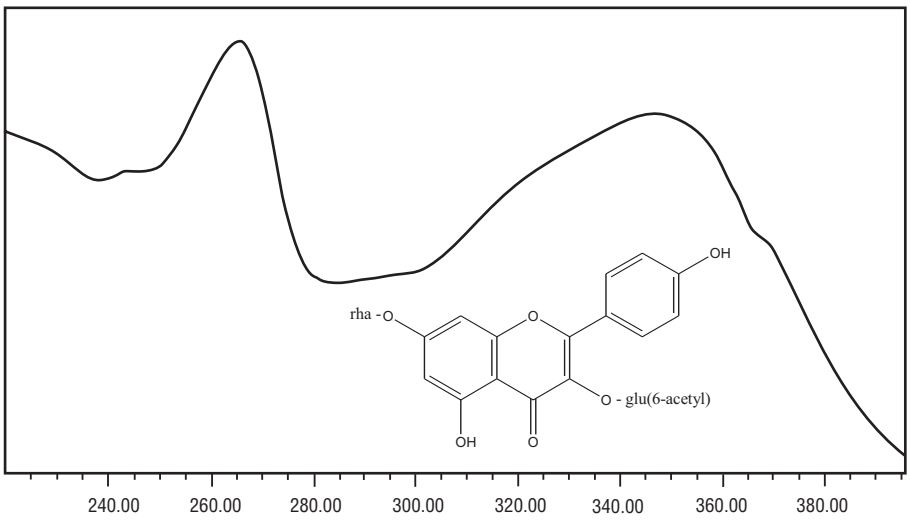


94 – kaempferol-3-*O*-[6-acetylglucoside]-7-*O*-glucoside

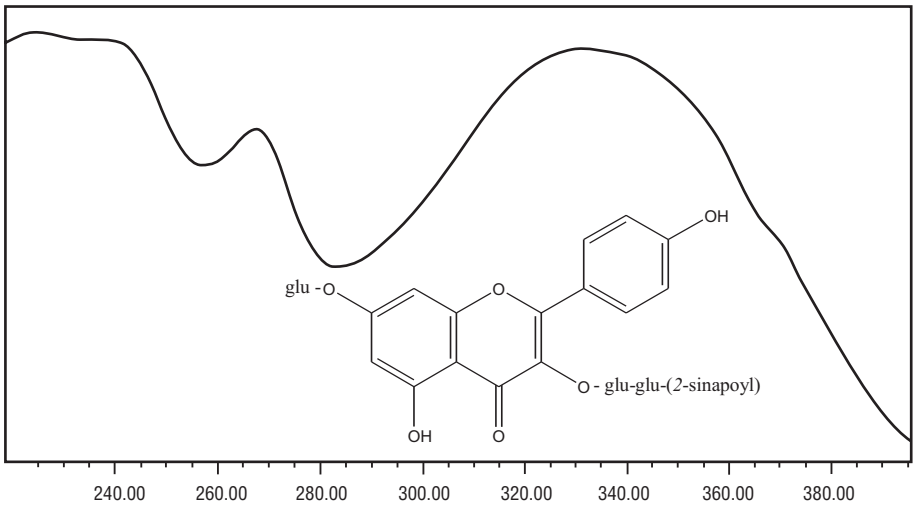
(RT = 30.1; $\lambda_{max.}$ = 242sh, 265, 315sh, 346 nm)



95 – **kaempferol-3-*O*-[6-acetylglucoside]-7-*O*-rhamnoside**
(RT = 34.3; $\lambda_{max.}$ = 242sh, 266, 315sh, 346 nm)

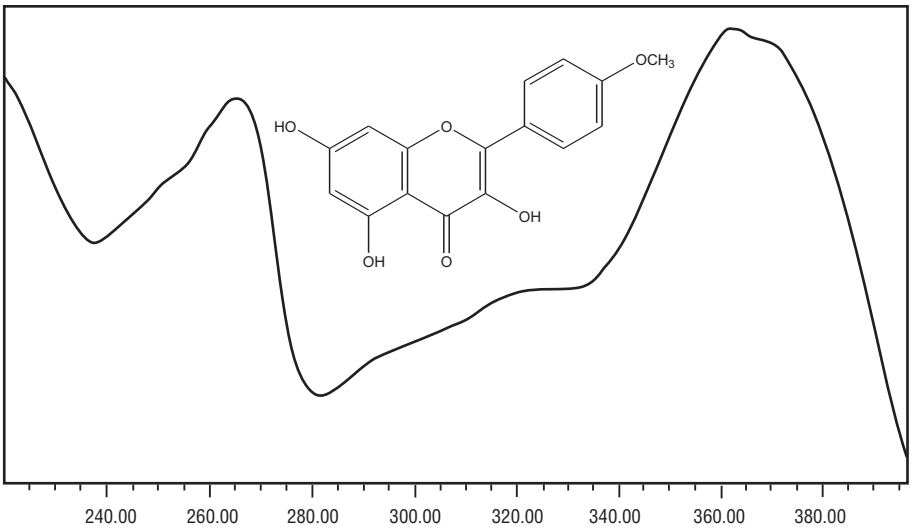


96 – **kaempferol-3-*O*-[2-sinapoylglucosyl(1-2)glucoside]-7-*O*-glucoside**
(RT = 26.8; $\lambda_{max.}$ = 226sh, 242sh, 268, 331 nm)



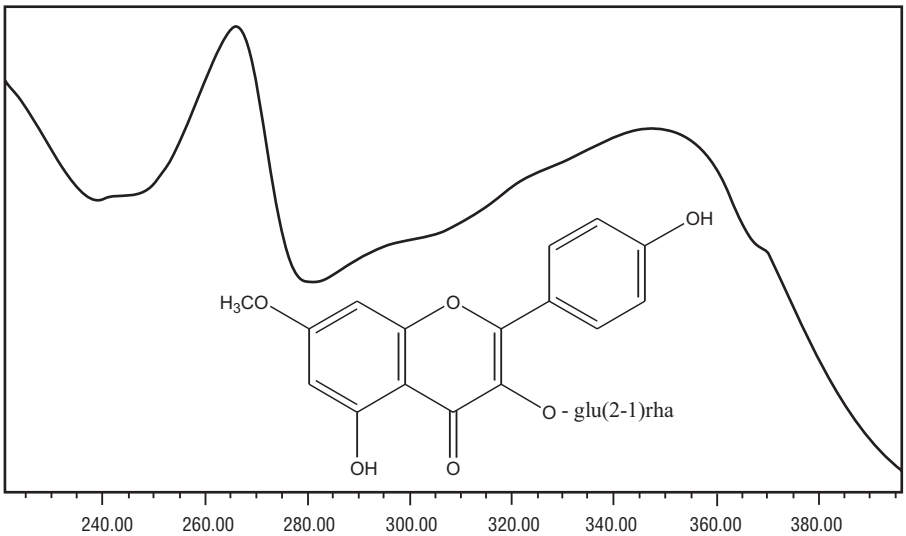
97 – **kaempferol-4'-*O*-methylether**

(RT = 58.2; $\lambda_{max.}$ = 248sh, 265, 295sh, 318sh, 362 nm)



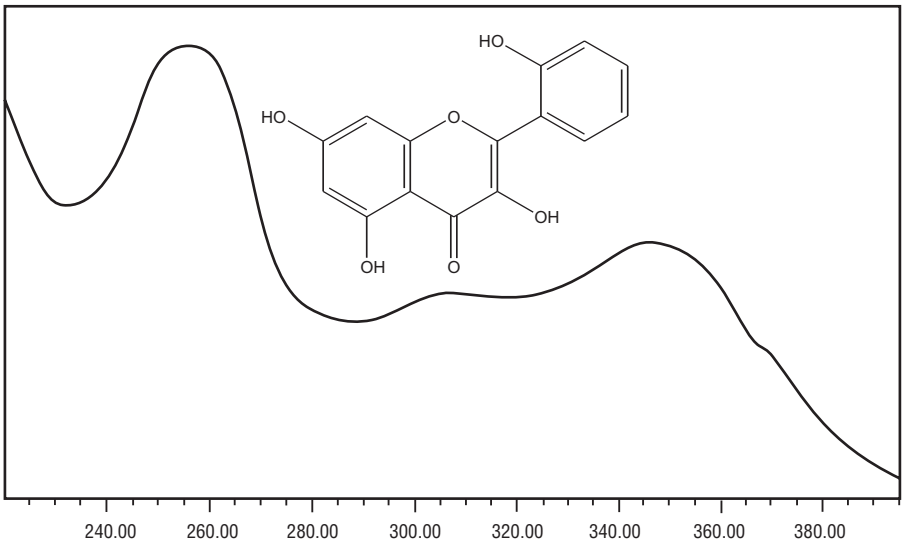
98 – 7-*O*-**methylkaempferol-3-*O*-[rhamnosyl(1-2)glucoside]**

(RT = 32; $\lambda_{max.}$ = 242sh, 263, 315sh, 344 nm)



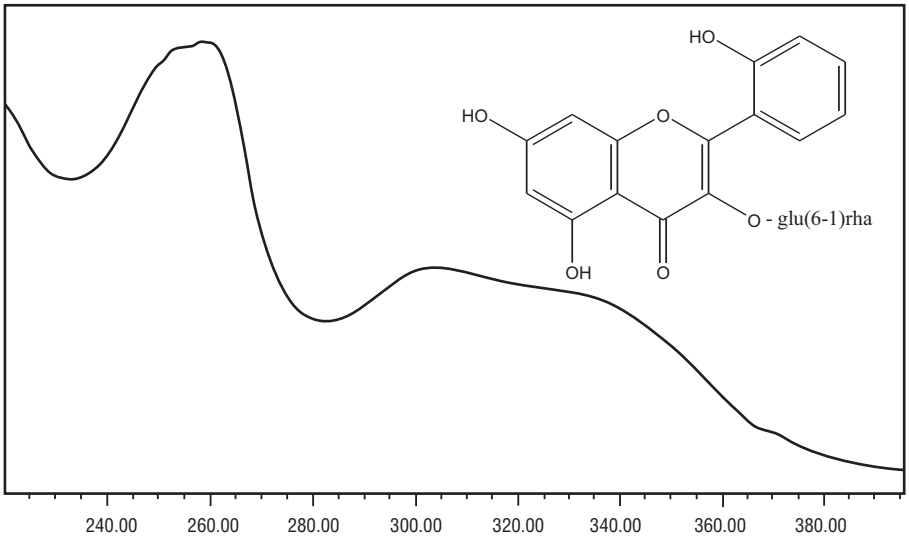
99 – **datiscetin**

(RT= 45.7; $\lambda_{max.}$= 255, 308sh, 346 nm)



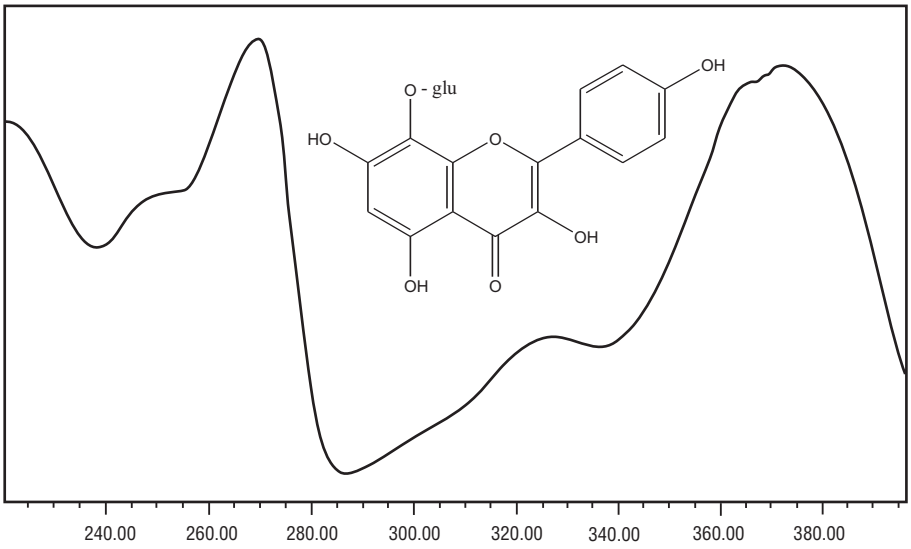
100 – **datiscetin-3-*O*-[rhamnosyl(1-6)glucoside]**

(RT=33.9; λ_{max} = 258, 304sh, 332sh nm)



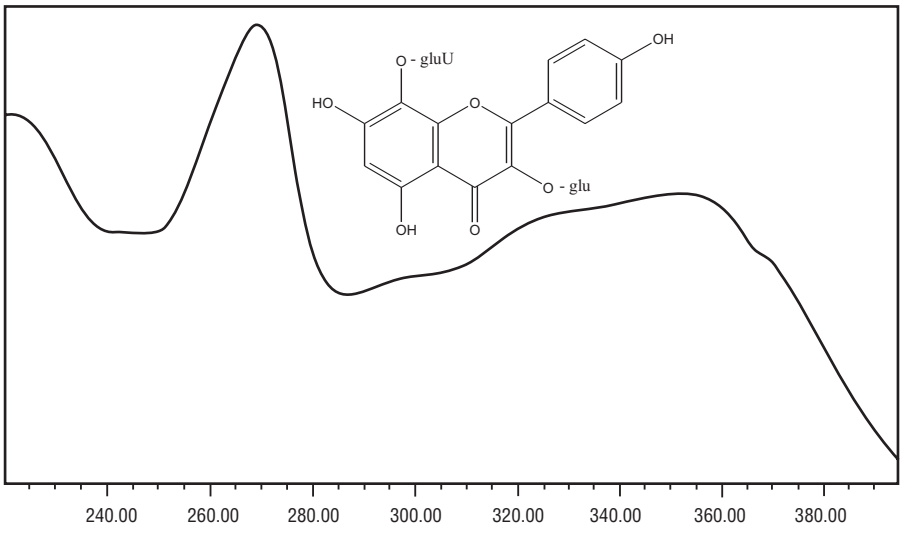
101 – herbacetin-8-*O*-glucoside

(RT = 38.5; $\lambda_{max.}$ = 250sh, 269, 326sh, 371 nm)



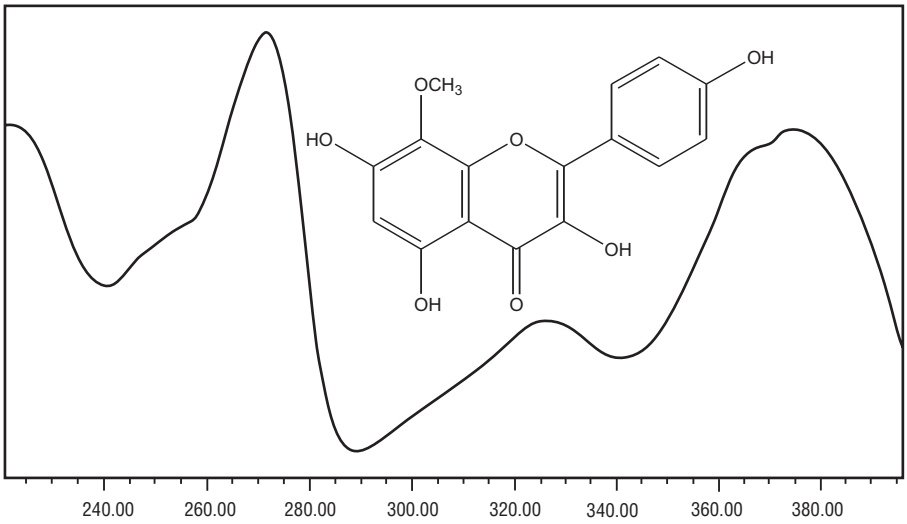
102 – herbacetin-3-*O*-glucosyl-8-*O*-glucuronide

(RT = 29.8; $\lambda_{max.}$ = 269, 295sh, 326sh, 352sh nm)



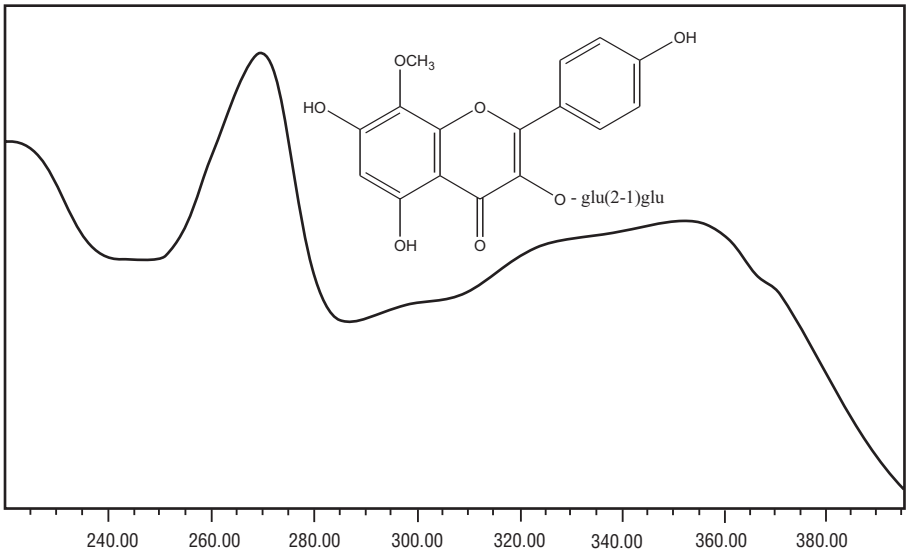
103 – 8-*O*-methylherbacetin

(RT = 46.3; $\lambda_{max.}$ = 250sh, 272, 325sh, 375 nm)

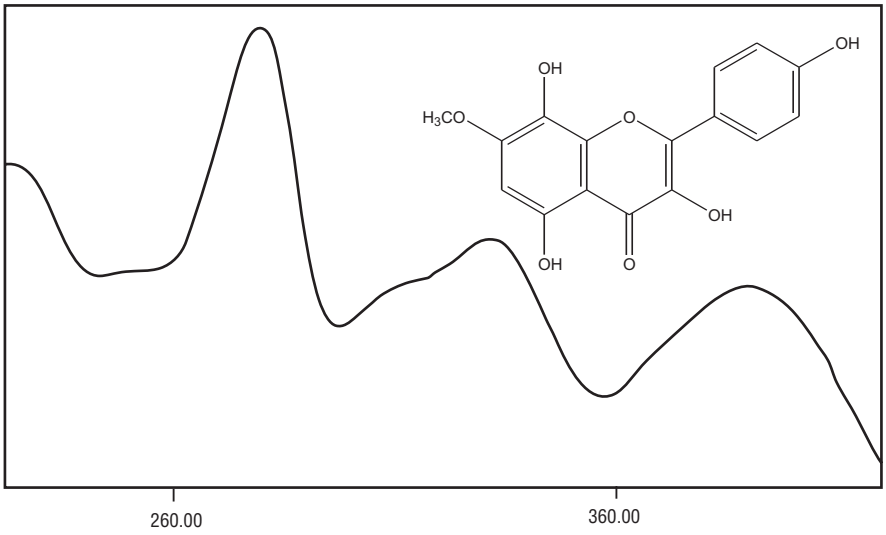


104 – 8-*O*-methylherbacetin-3-*O*-[glucosyl(1-2)glucoside]

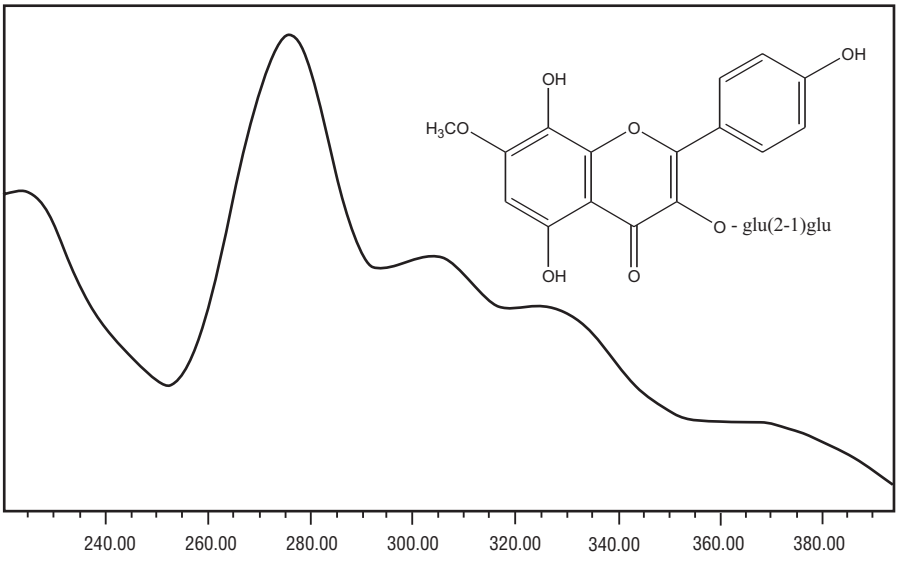
(RT = 31.4; $\lambda_{max.}$ = 274, 300sh, 324sh, 355sh nm)



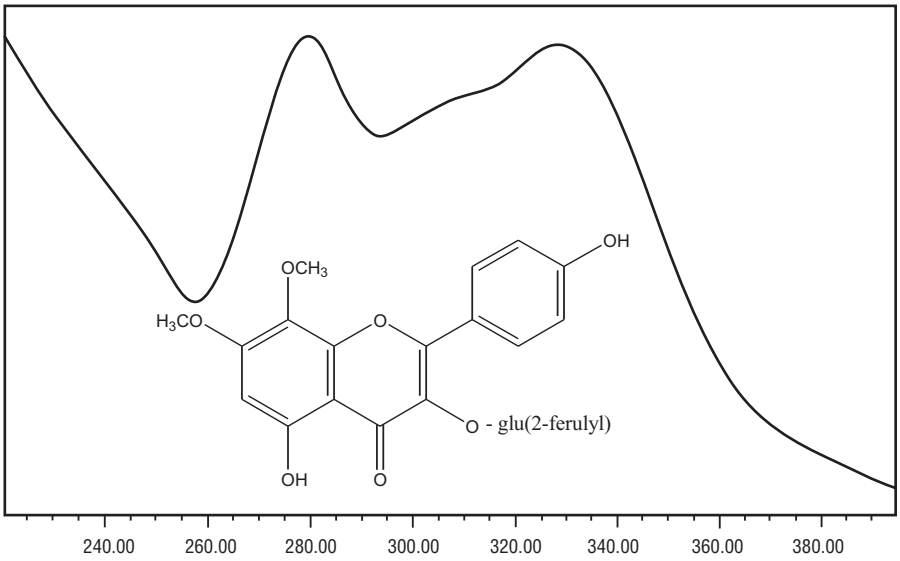
105 – 7-*O*-**methylherbacetin**
(RT = 45.6; $\lambda_{max.}$ = 276, 307sh, 329, 387 nm)



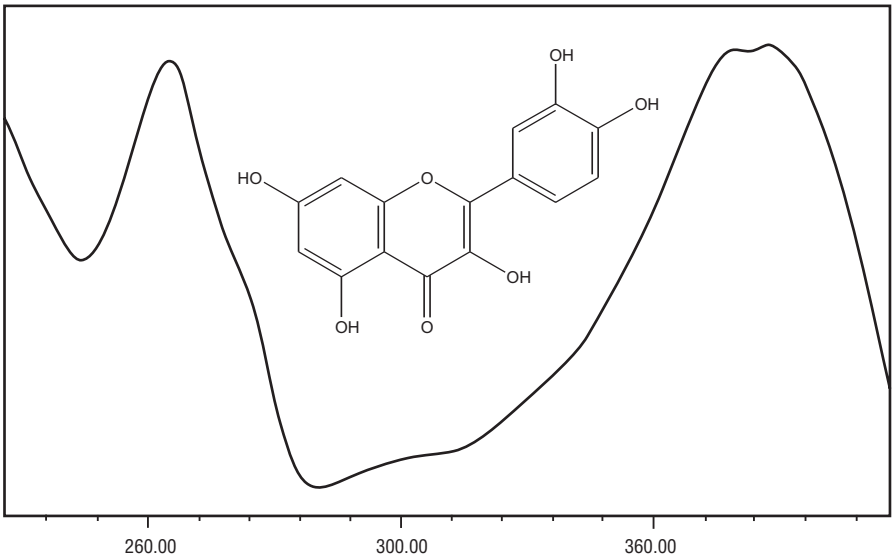
106 – 7-*O*-**methylherbacetin-3-*O*-[glucosyl(1-2)glucoside]**
(RT = 30.9; $\lambda_{max.}$ = 278, 307, 330, 370sh nm)



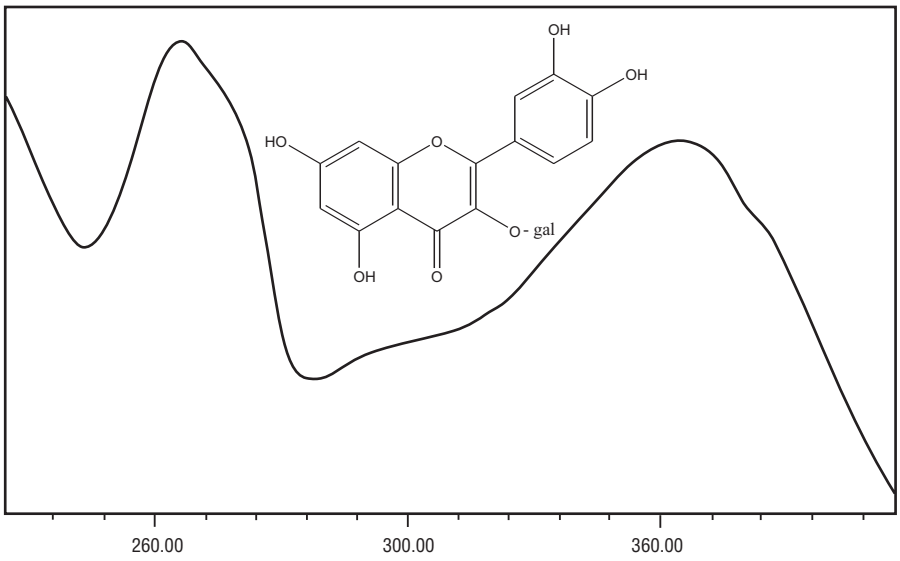
107 – 7,8-di-*O*-**methylherbacetin-3-*O*-[2-ferulylglucoside]**
(RT = 43; $\lambda_{max.}$ = 279, 310sh, 329 nm)



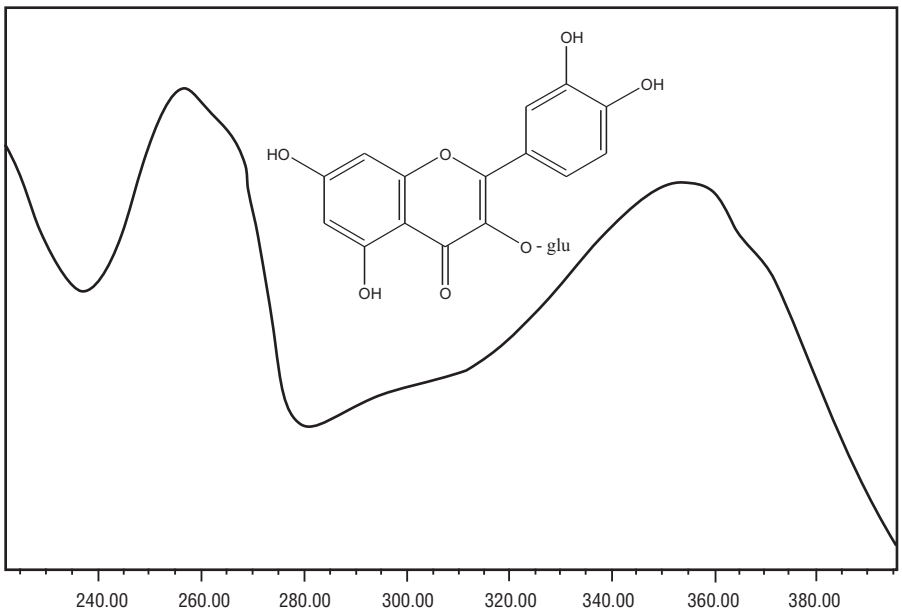
108 – **quercetin**
(RT = 41.9; $\lambda_{max.}$ = 255, 295sh, 372 nm)



109 – **quercetin-3-*O*-galactoside**
(RT = 33.5; $\lambda_{max.}$ = 255, 266sh, 294sh, 355 nm)

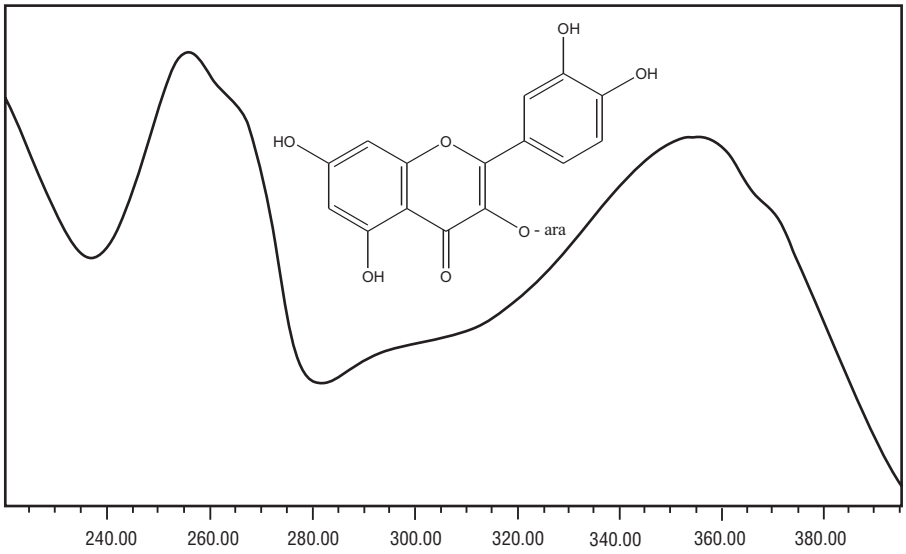


110 – **quercetin-3-*O*-glucoside**
(RT = 33.8; $\lambda_{max.}$ = 255, 266sh, 294sh, 355 nm)



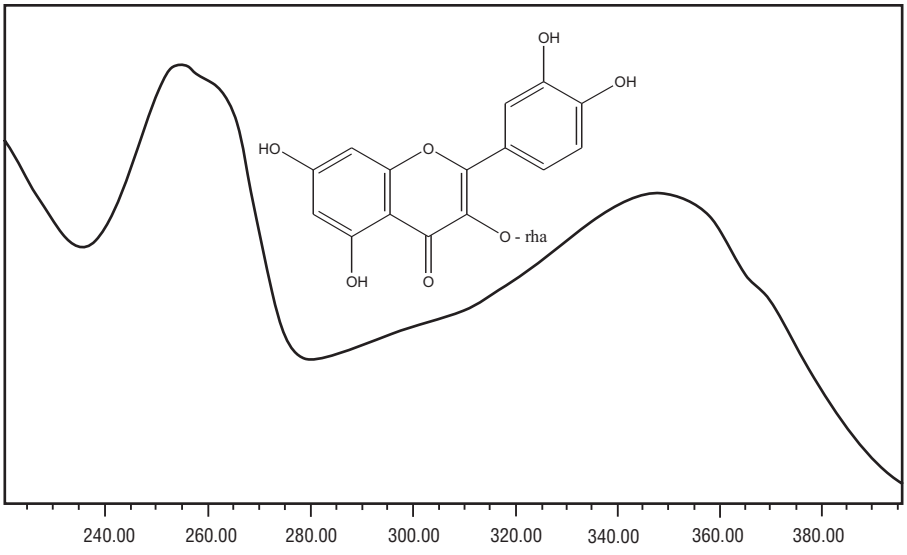
111 – **quercetin-3-*O*-arabinoside**

(RT= 34.9; $\lambda_{max.}$ = 255, 266sh, 294sh, 354 nm)



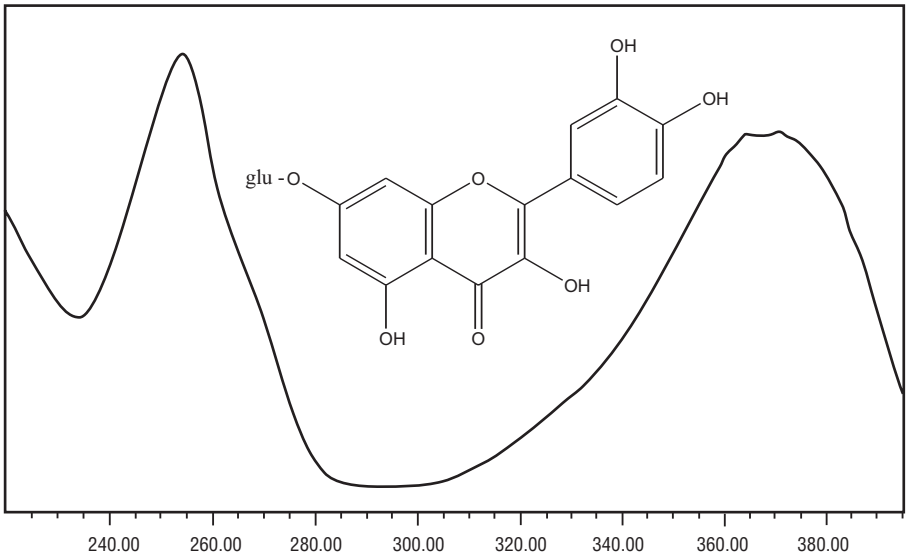
112 – **quercetin-3-*O*-rhamnoside**

(RT = 35.7; $\lambda_{max.}$ = 254, 263sh, 294sh, 348 nm)



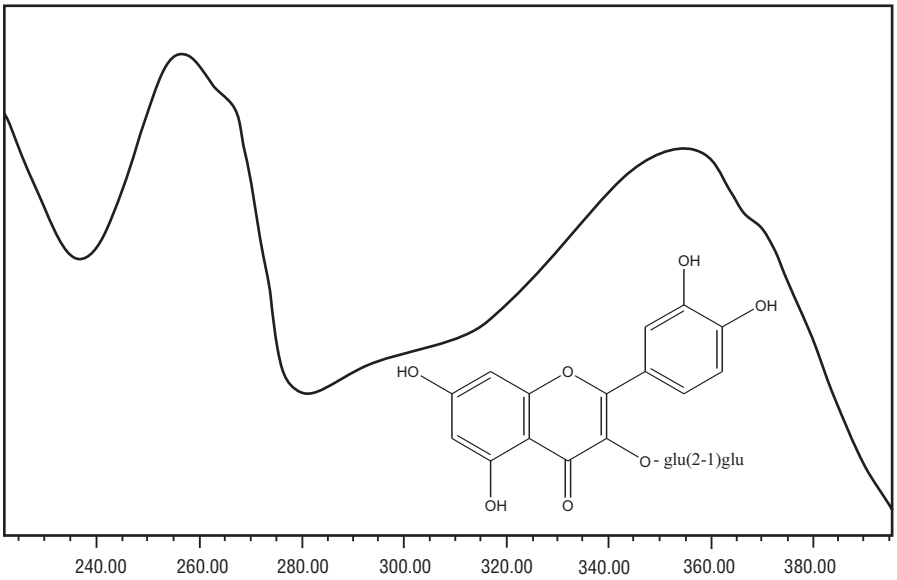
113 – quercetin-7-*O*-glucoside

(RT = 32.6; $\lambda_{max.}$ = 254, 269sh, 370 nm)

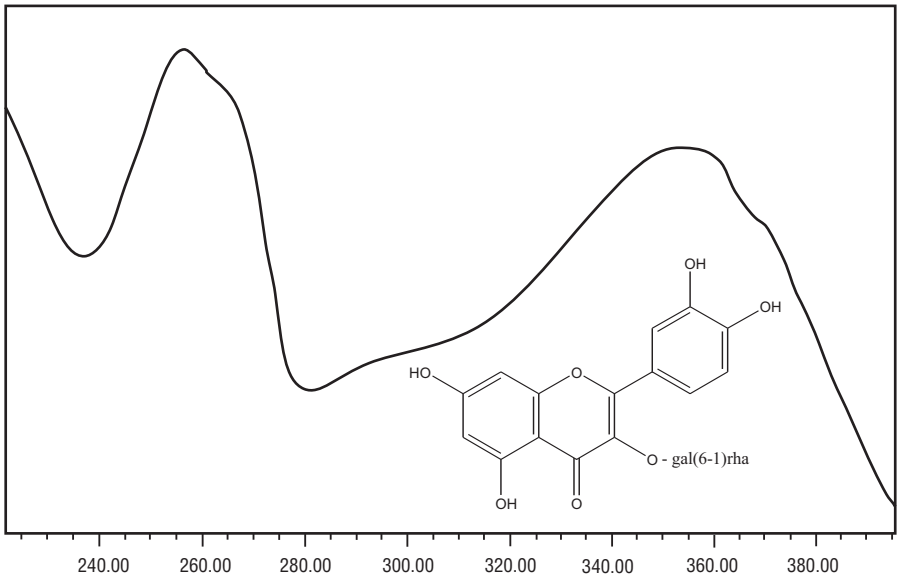


114 – quercetin-3-*O*-[glucosyl(1-2)glucoside]

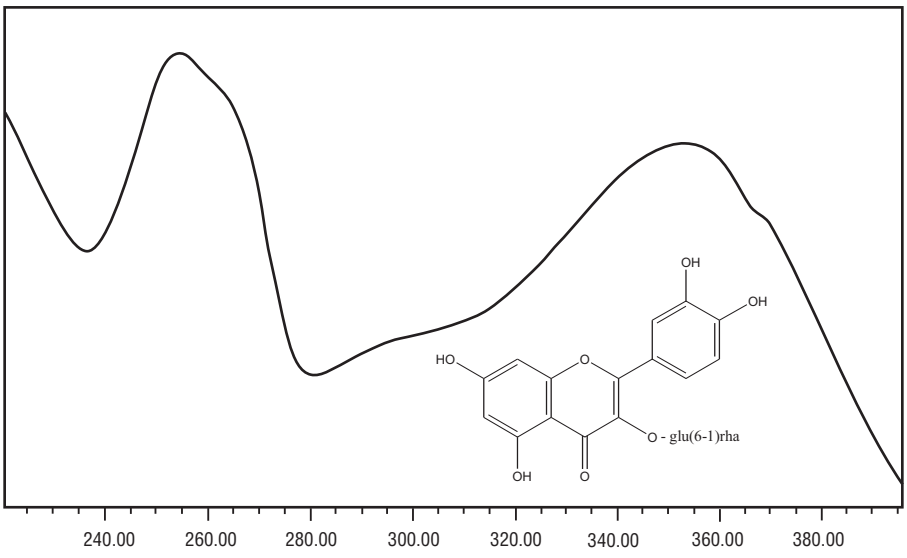
(RT = 30.6; $\lambda_{max.}$ = 255, 266sh, 294sh, 355 nm)



115 – **quercetin-3-*O*-[rhamnosyl(1-6)galactoside]**
(RT = 32.7; $\lambda_{max.}$ = 255, 266sh, 294sh, 355 nm)

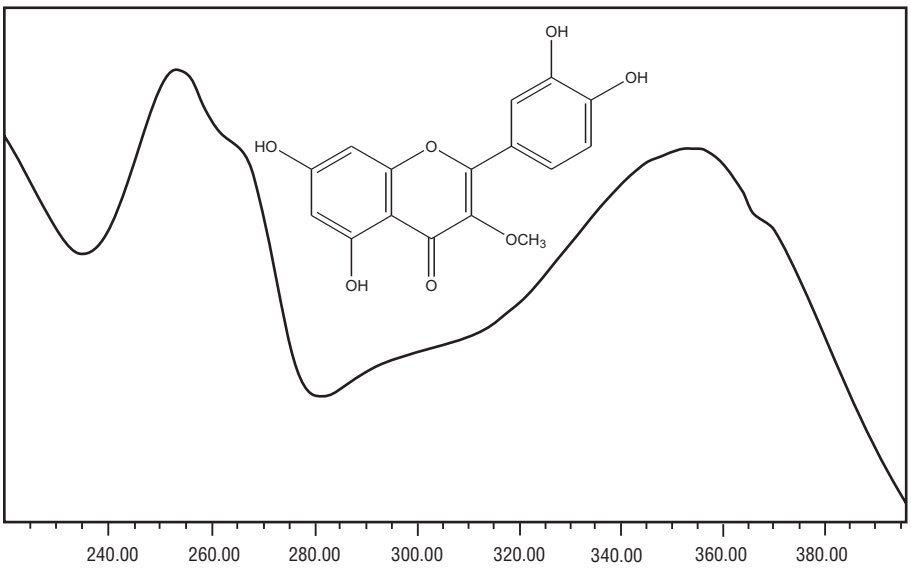


116 – **quercetin-3-*O*-[rhamnosyl(1-6)glucoside]**
(RT = 33.1; $\lambda_{max.}$ = 255, 264sh, 294sh, 355 nm)



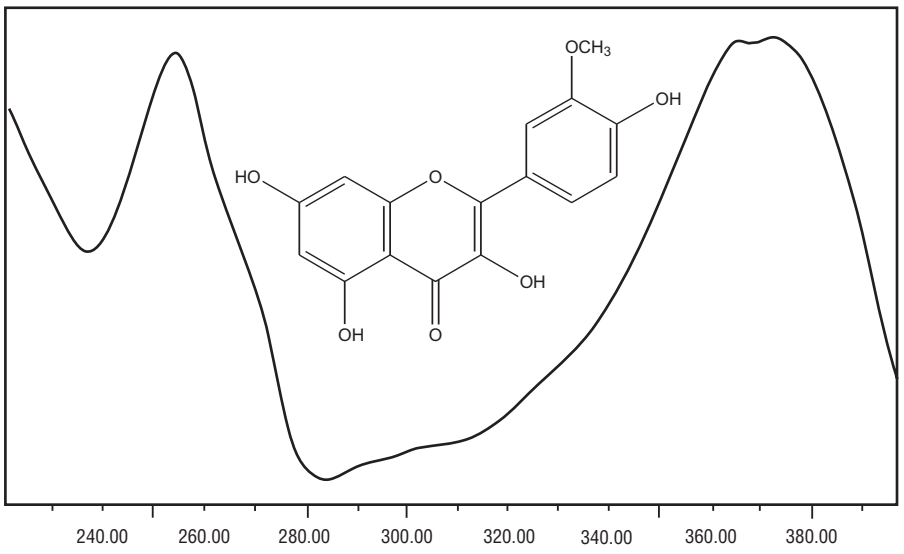
117 – 3-*O*-methylquercetin

(RT = 43.4 ; $\lambda_{max.}$ = 255, 263sh, 297sh, 357 nm)



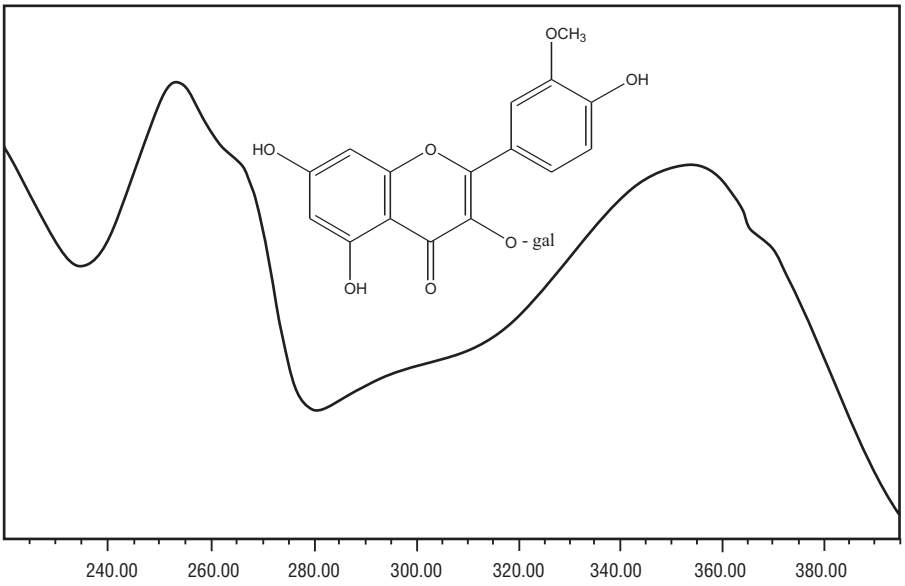
118 – isorhamnetin

(RT = 47.3; $\lambda_{max.}$ = 255, 266sh, 306sh, 326sh, 372 nm)



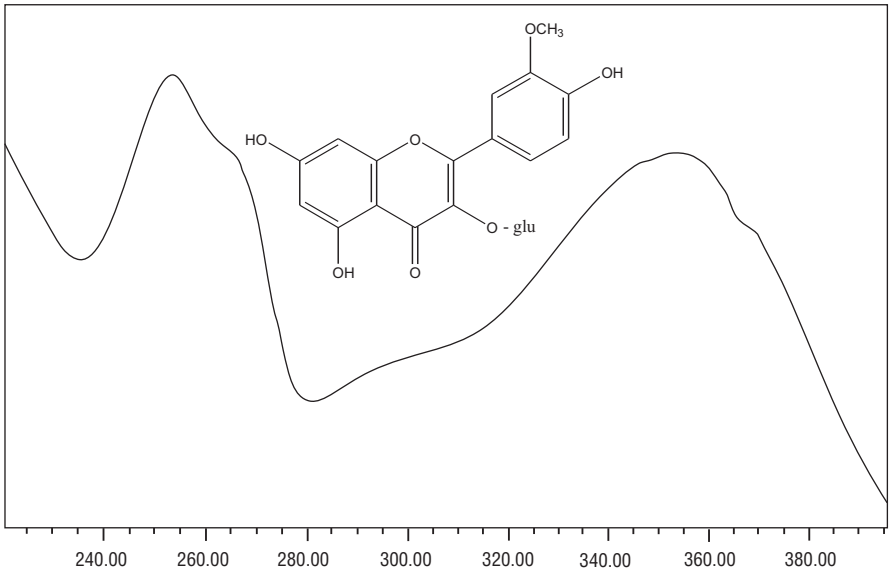
119 – **isorhamnetin-3-*O*-galactoside**

(RT = 36; $\lambda_{max.}$ = 253, 266sh, 294sh, 352 nm)



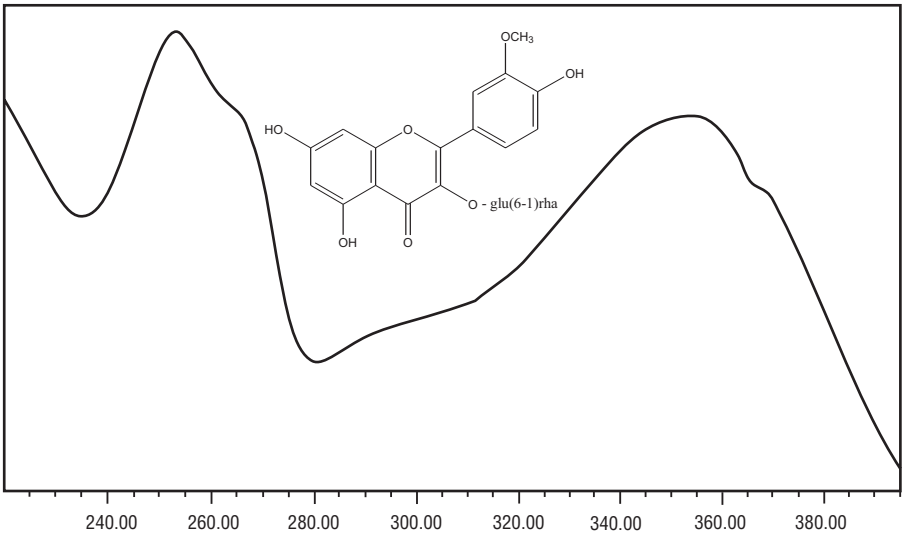
120 – **isorhamnetin-3-*O*-glucoside**

(RT = 36.3; $\lambda_{max.}$ = 255, 266sh, 294sh, 355 nm)



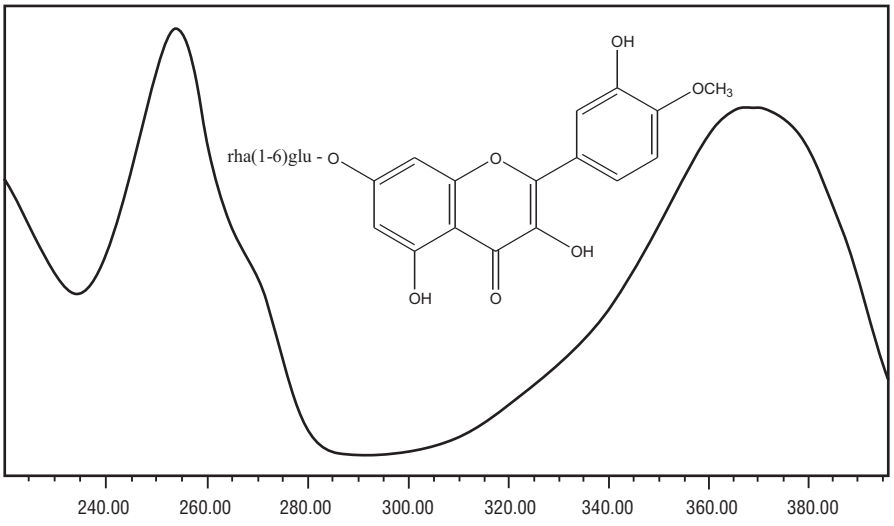
121 – isorhamnetin-3-*O*-[rhamno(1-6)glucoside]

(RT = 35.5; $\lambda_{max.}$ = 255, 266sh, 294sh, 355 nm)



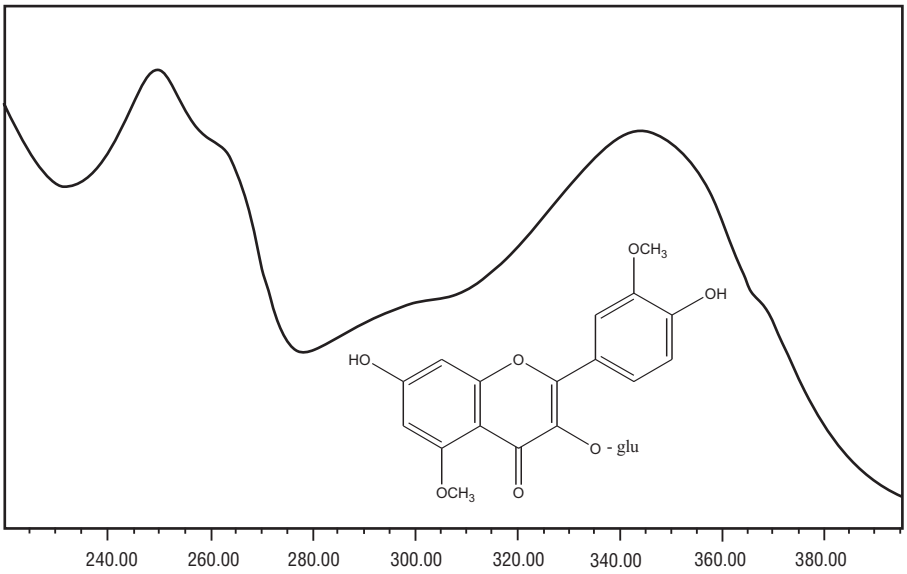
122 – tamarixetin-7-*O*-rutinoside

(RT = 35.9; $\lambda_{max.}$ = 253, 269sh, 369 nm)



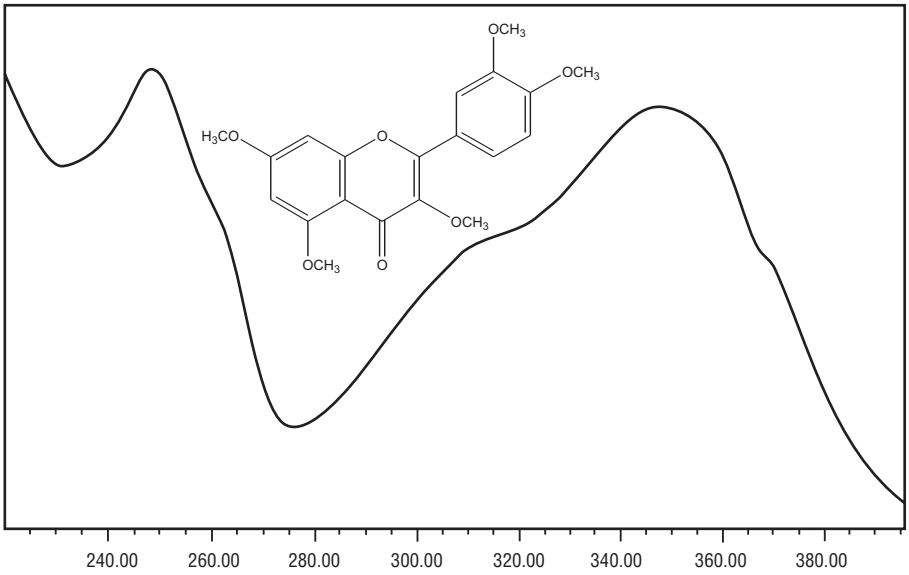
123 – 5-*O*-methylisorhamnetin-3-*O*-glucoside

(RT = 33.8; $\lambda_{max.}$ = 249, 263sh, 298sh, 344 nm)



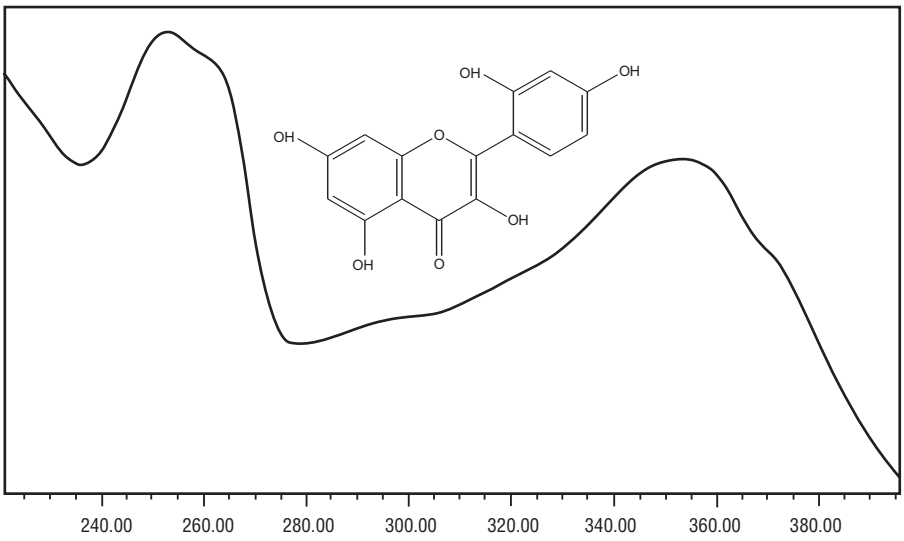
124 – penta-*O*-methylquercetin

(RT = 55.5; $\lambda_{max.}$ = 248, 262sh, 318sh, 346 nm)



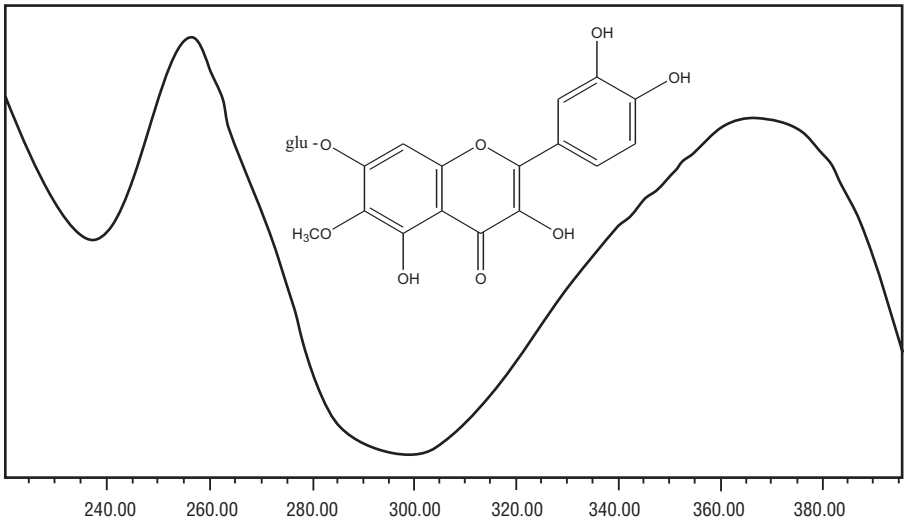
125 – morin

(RT = 39.5; $\lambda_{max.}$ = 252, 262sh, 290sh, 318sh, 352 nm)



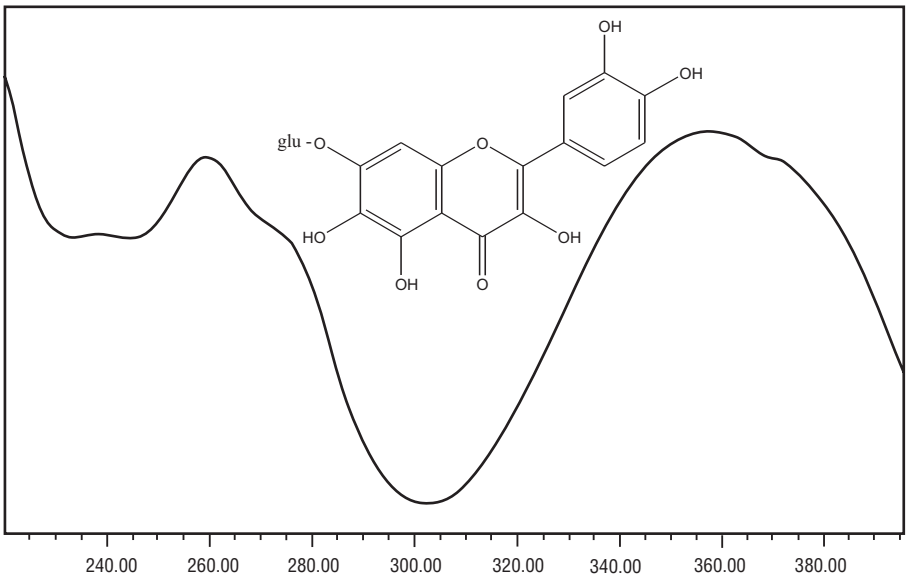
126 – patuletin-7-*O*-glucoside

(RT = 33.5; $\lambda_{max.}$ = 256, 272sh, 372 nm)



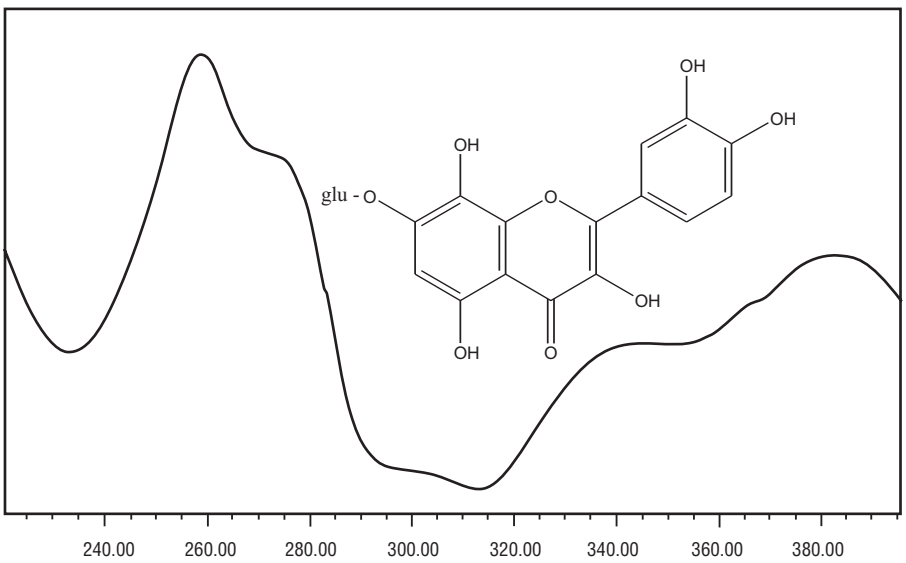
127 – quercetagenin-7-*O*-glucoside

(RT = 30.7; $\lambda_{max.}$ = 236sh, 259, 274sh, 358 nm)



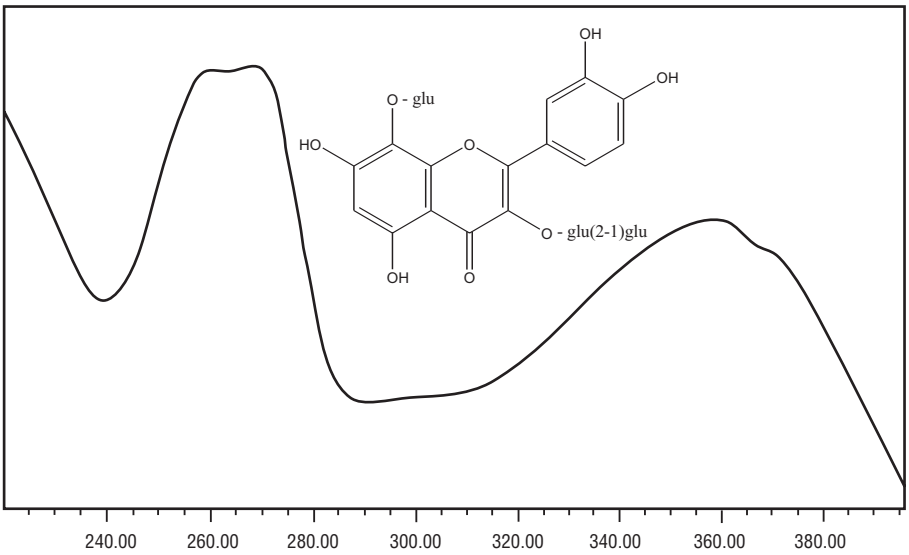
128 – gossypetin-7-*O*-glucoside

(RT = 31.8; $\lambda_{max.}$ = 259, 269sh, 300sh, 340sh, 381 nm)



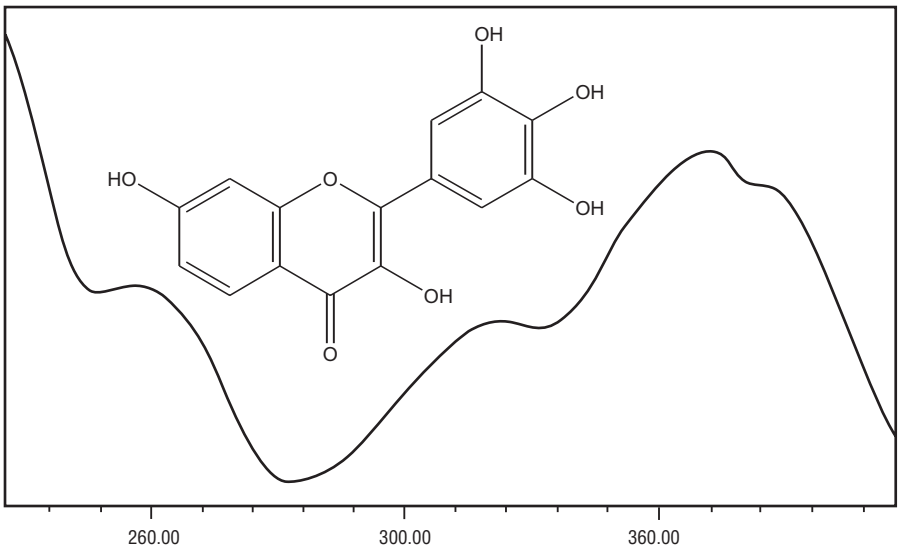
129 – gossypetin-8-*O*-glucoside-3-*O*-[glucosyl(1-2)glucoside]

(RT = 24.6; $\lambda_{max.}$ = 259sh, 268, 356 nm)



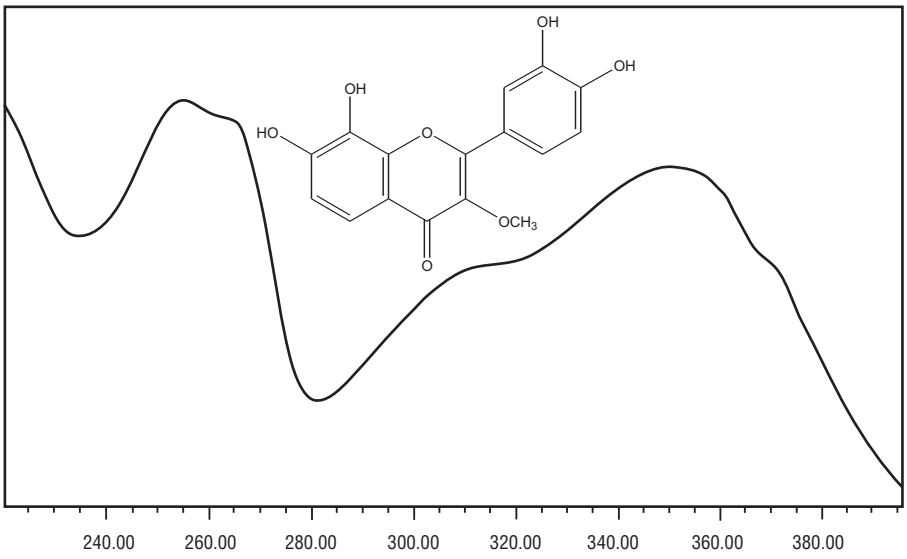
130 – robinetin

(RT = 33.7; $\lambda_{max.}$ = 246, 316, 358 nm)



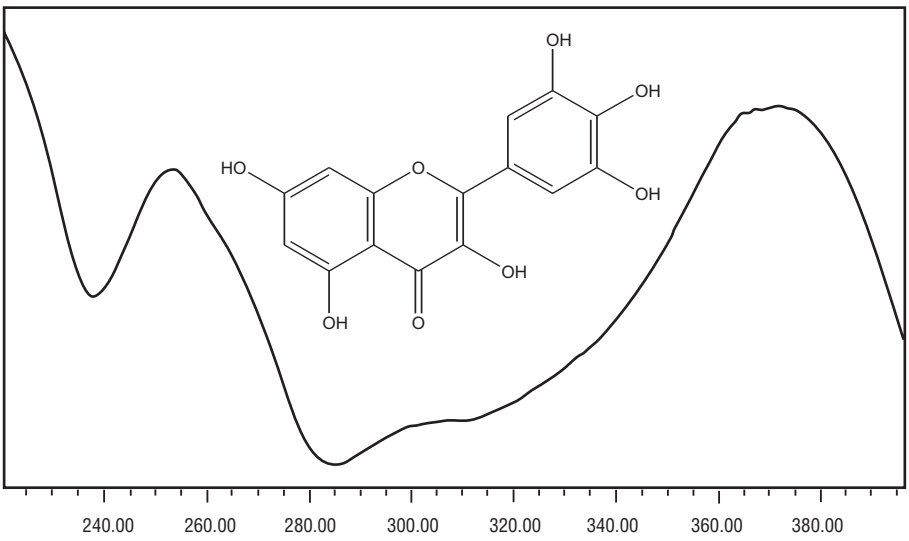
131 – 3-*O*-methyl-7,8,3',4'-tetrahydroxyflavonol

(RT = 33.2; $\lambda_{max.}$ = 255, 265sh, 310sh, 345 nm)



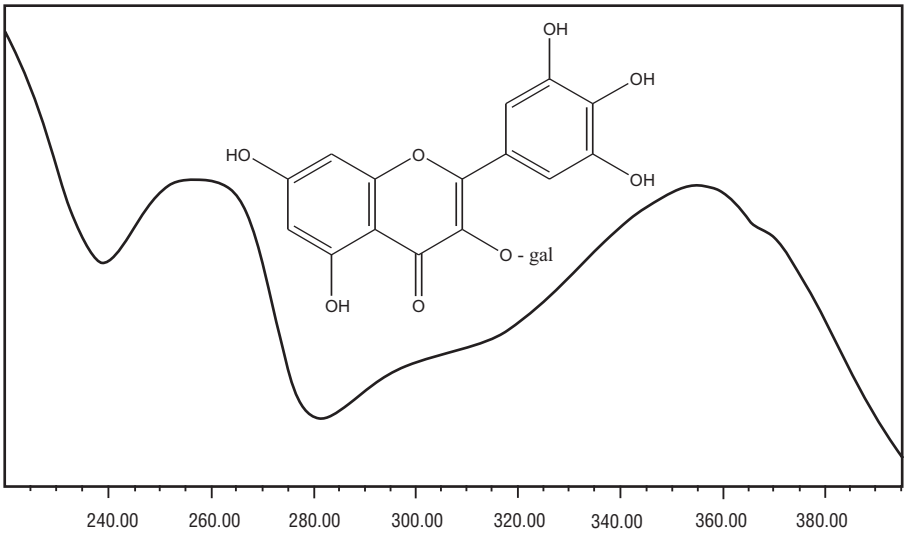
132 – myricetin

(RT = 37.2; $\lambda_{max.}$ = 252, 263sh, 298sh, 371 nm)



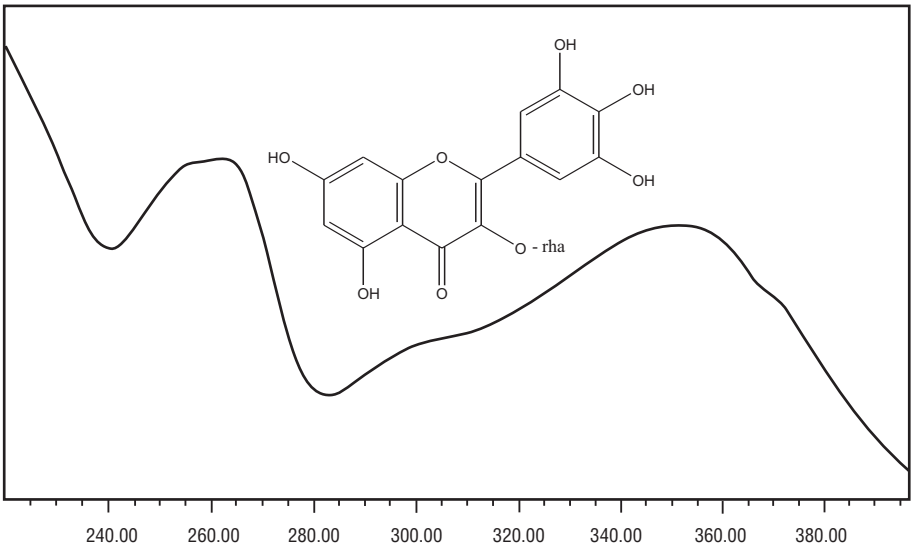
133 – myricetin-3-*O*-galactoside

(RT = 30.8; $\lambda_{max.}$ = 250, 261sh, 300sh, 354 nm)



134 – myricetin-3-*O*-rhamnoside

(RT = 32.8; $\lambda_{max.}$ = 250sh, 261, 300sh, 349 nm)



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Série

Ensino

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