Effect of flavonoïds from *Arbutus unedo* leaves on rat isolated thoracic aorta

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Abstract: *Arbutus unedo* (*A. unedo*) is a medicinal plant commonly used to treat hypertension and diabetes in Oriental Morocco. Our previous studies showed that *A. unedo* has a vascular, diuretic, natriuretic, antiagregant and antidiabetic properties. Our goal was to show the vascular action of two groups of flavonoids (free and heterosidic flavonoids) extracted from the leaves of *A. unedo* on the isolated aorta of rat. *A. unedo* leaves were collected from Tazekka Mountain (Morocco). Two flavonoïds-enriched (Genins and Heterosids) and a third final aqueous fractions were obtained using the soxhlet refluxing apparatus and tested *in vitro* on a phenylephrine-precontracted aorta. Thin layer chromatography analysis of both flavonoïds-enriched fractions was performed with silica gel on an aluminum support. The determination of total polyphenolic was achieved using Folin-Ciocalteu method and the total content of flavonoids was determined by aluminum chloride method. The genins-enriched fraction induced a moderate contraction of aorta while the aqueous fraction caused an opposite effect. The heterosids-enriched fraction induced two distinct effects, a relaxation followed by a contraction of the same amplitude. All these effects are endothelium-dependent but not depending on each another. Moreover, the determination of total polyphenolic and flavonoid contents and thin layer chromatography analysis confirmed the abundance of these compounds in the studied fractions of *A. unedo* leaves. In conclusion, the vasorelaxant property of aqueous and heterosidic fractions are partly attributed to the presence of the flavonoids compounds. These results confirm the validity of the traditional use of *A. unedo* in hypertension treatment

Keywords: *Arbutus unedo*, Ericaceae, Genins; Heterosids, Polyphenols, Vascular effect.1.

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1.Introduction

Arbutus unedo L. (Ericaceae) is one of the most commonly used medicinal plants in Oriental Morocco to treat several diseases such as hypertension and diabetes (Ziyyat et al. 1997). Several studies carried out in our laboratory showed that *Arbutus unedo* (*A. unedo*) has a vascular, diuretic, natriuretic (Ziyyat & Boussairi 1998; Ziyyat et al. 2002; Legssyer et al. 2004), antiagregant (Mekhfi et al. 2006) and antidiabetic properties (Bnouham et al. 2010). The vascular effect of this plant was endothelium-dependant and could be attributed to polyphenolic compounds principally condensed tannins and catechin gallate (Legssyer et al. 2004). Beside tannins, other components may be involved in this effect such as flavonoids which are abundant in *A. unedo* leaves (Dauget & Foucher 1982). Recently, it was found that *A. unedo* leaves and roots aqueous extract reduce the development of blood pressure and improve cardiovascular and renal structural (unpublished data) and functional changes induced by chronic inhibition of NO synthesis by L-NAME in rat (Afkir et al. 2008). Other studies have shown that *A. unedo* has an antioxidant; antimicrobial (Malheiro et al. 2012; Dib et al. 2013; Djabou et al. 2013) anti-inflammatory (Mariotto et al. 2008a; 2008b) and anticancer effects (Carcache-Blanco et al. 2006). These beneficial effects may justify the traditional use of this plant for treating hypertension.

The phytochemical studies show the richness of *A. unedo* in polyphenolic compounds mainly tannins and flavonoids such as Quercitrin; Isoquercitrin, Hyperoside, Rutin (Dumon et al. 1996; Males et al. 2013), Afzelin; Juglanin; Avicularin; Quercitrosid and Myricetin of glucosids (Dauget & Foucher 1982; Fiorentino et al. 2007). For that reason, we focused our attention on flavonoids compounds that are more abundant in *A. unedo* leaves. In fact, flavonoids are the most common group of plant polyphenols. They have gained an increased interest with regard to their potential in cardiovascular protection (Scalbert et al. 2005; Alam et al. 2013; Yamagata et al. 2014). In fact, the flavonoids exhibit wide range of biological effects including inhibition of platelet aggregation, antioxidant and anti-inflammatory properties and modulate the vascular tone (Middleton et al. 2000; Fusi et al. 2003; Pastore et al. 2012; Almeida Rezende et al. 2015).

The aim of the present investigation was to show the effect of two groups of flavonoids (free and heterosidic flavonoids) extracted from the leaves of *A. unedo* on the isolated thoracic aorta of rat.

2. Materials and Methods

2.1. Animals

Adult male and female Wistar rats, weighing 250 to 320g, were used in this study. They were obtained from our local colonies maintained at our department. They were kept under conditions of constant temperature (22 ±2°C) with a standard light-dark cycle (12h light /12h dark) and free access to food and water. All animals were cared for in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication 85-23, revised 1996; see the following web site http://www.nap.edu/readingroom/books/labrats/index.html).

2.2. Plant material

A. unedo leaves were collected on May 2006 from Tazekka Mountain (Morocco). Taxonomic identification was performed by Pr B. Haloui (Biology Department of Sciences Faculty, University Mohamed First, Oujda, Morocco). Voucher specimen was deposited in the herbarium of the same department with number collection ZL 14.

2.3. Extraction of flavonoïds

200g of dried and powdered leaves of *A. unedo* were firstly degreased with petroleum ether (450ml) using the soxhlet refluxing apparatus for 10h. After filtration, the degreased vegetal material has undergone an extraction under reflux for 10h with the mixture acetone/ water 1/1.5. After filtration with Buchner, the filtrate is evaporated in *vacuum* using rotary evaporator to remove acetone. The aqueous solution was recovered again; washed with petroleum ether (2 ×100ml) by decantation in order to remove all lipids and chlorophylls. For the extraction of the genins (or free flavonoïds), the recovered aqueous phase was washed with diethyl ether (3 ×100ml). The remaining aqueous fraction was washed afterwards with ethyl acetate (3 ×100ml) to isolate the heterosidic flavonoïds (Mekhfi et al. 2006). The amount in dried extract of such fraction calculated in mg/100g of starting dried plant material was 0.4% and 2.7% respectively for genin and heterosidic enriched fractions and it was 18.3% and 6.8% for final aqueous solution and lipid fraction respectively.

2.4. Preparation of rat thoracic aorta rings

The rats were anesthetized with sodium pentobarbital (50mg/kg of body weight, i.p.) and the thoracic aorta was removed carefully and immersed in fresh KHB solution (pH 7.4) of the following composition (mM): NaCl 119, KCl 4.7, $MgCl_2$ 1.2, $CaCl_2$ 1.6, KH_2PO_4 1.2,

$NaHCO_3$ 25, and glucose 11.1. After removing adhering fat and connective tissues, the aorta was cut into cylindrical strips of about 2-3mm in length, which were suspended between two stainless steel hooks in a 11 ml organ baths containing continuously aerated (95% O_2 and 5% CO_2) perfusion medium at 37°C (pH 7.4). Strips were stretched with a passive tension of 1 g and tension was recorded using an isometric transducer (EMKA Technologies, Paris, France) connected to a paper recorder (Leybold-Heraeus, type SE122).

After an equilibration period of 30 to 45min, each preparation was challenged at the beginning of the experiment with a sub-maximal concentration (1µM) of phenylephrine (PHE). Once a sustained contraction was established, carbachol (100µM) was added to the bath and the presence of carbachol-induced vasorelaxant effects was taken as evidence that the vessel segment had a functional and intact endothelium. In order to obtain denuded aorta, the endothelium was removed mechanically by rubbing the lumen of the artery with plastic tubing. The absence of relaxation in response to Cch was taken as evidence that the vessel segments were successfully denuded of endothelium. Thereafter, concentration-response curves to increasing concentrations (from 10^{-4} to 10^{-1}g/l) of such fraction (genin, heterosid and aqueous) were constructed in a cumulative manner in the presence and absence of endothelium. Relaxation data were expressed as a percentage of the maximal phenylephrine-induced contraction.

2.5. Determination of total polyphenolic content

The determination of total polyphenolic content was achieved spectrometrically using Folin-Ciocalteu method described by Hagerman et al. (Hagerman et al. 2000). The dry extract of each fraction was solubilised in distilled water, and to aliquots of 0.5ml were added 0.25ml of Folin–Ciocalteu reagent and 1.25ml of aqueous sodium carbonate solution at 20%; samples were vortexes and kept in the dark for 40min. The blank is prepared in the same conditions as assay tubes. The presence of polyphenols was revealed by the appearance of a blue color and absorbance was measured at 725nm using a spectrophotometer (Spectronic ® 20 Genesys ™ Spectrophotometer, USA). The amount of total polyphenols was calculated as a catechin equivalent from the calibration curve of 6-catechin standard solutions and expressed in mg equivalent of catechin per 100g of dry plant material. Each measurement was repeated four times.

2.6. Flavonoïds determination

The aluminum chloride method ($AlCl_3$) was used to determine the total content of flavonoids extracts (Jay et al. 1975). To each 5ml of test solution, 2.5ml of $AlCl_3$ reagent were added (133mg crystalline aluminum chloride and 400mg crystalline sodium acetate were dissolved in 100ml of extracting solvent). The mixture was kept in the dark for 40min. After this period, a yellow color develops and absorbance was measured at 430nm against a blank (5ml of analyzed solution plus 2.5ml of water). Quantification of flavonoids was made with a standard range achieved in the presence of rutin as standard. The flavonoids content was expressed as mg of rutin/100g of starting dry plant material. Measurements were repeated four times.

2.7. Thin layer chromatography of flavonoïds

The thin layer chromatography analysis was performed with silica gel on an aluminum support (thickness = 0.2mm, 20x20cm). Both fractions rich in flavonoids (heterosidic and genin) were dissolved in their solvent of extraction. The solution was deposited on the chromatography plate using a capillary. After drying, the plate is placed in a chromatography tank containing the mobile phase and its saturated vapor. After migration, the plate is dried and then evaluated under UV light at 254 and 366nm. To confirm the presence of flavonoids, the chromatograms were additionally sprayed with the crystallized aluminum chloride ($AlCl_3$) and/or the anisaldehyde/H_2SO_4 and then heated at 100°C for a few min. The separation of heterosidic flavonoids from the aglyconic flavonoids was done using the α-naphthol which is a specific indicator of sugars by the appearance of purple coloration. To achieve a good separation of the genin enriched fraction, we tested several mobile phases listed in table 1. The best result is obtained with the phase 10 (benzene-methanol-acetic acid (37:12:1, v/v/v)). For the separation of the polar constituents of heterosidic enriched fraction, we have improved the polarity of the mobile phase 10 by increasing the percentage of methanol and decreasing that of benzene. The different mobile phases used were: Benzene-methanol-acetic acid: 29/20/1, 25/24/1, 16/33/1 and 12/37/1. The best result is obtained with the mixture benzene-methanol-acetic acid (29:20:1, v/v/v).

2.8. Statistics

Data obtained were analyzed using Student's t-test and were presented as mean ± SEM. The concentration-response curves of phenylephrine and carbachol were analyzed by nonlinear regression using GraphPad Prism V 4.0 software. Differences were considered significant for $p<0.05$.

3. Results and discussion

3.1. Results

3.1.1. Effect of genin enriched fraction on response of the aorta rings

Figure 1 shows the original tracings (A1 and A2) and the concentration-response of the genin fraction on PHE (1µM) pre-contracted thoracic aorta ring with or without endothelium (A3). The addition of cumulative concentrations ranging from 10^{-4} to 10^{-1}g/l of the genins potentiated the contractile response to PHE, which was attenuated by removal of the endothelium. The vasoconstriction of intact aorta (in the presence of functional endothelium) induced by the genin fraction was concentration-dependent with a maximal contraction Cmax =0.3 ± 0.04g (n=7) at 10^{-1}g/l.

3.1.2. Effect of the heterosidic enriched fraction on vascular response

The test of increasing concentrations of the heterosidic fraction on aortic rings with functional endothelium pre-contracted to PHE (1µM), induced a potent relaxation of the muscle at the concentration of 10^{-2} g/l with a maximum of relaxation Rmax= 77 ± 2% (n =7) (Fig. 2B1 and B3). This vasorelaxant effect disappeared on aortic ring without endothelium (Fig. 2B2 and B3), suggesting that the vasodilator effect obtained is endothelium-dependent. Whereas, the concentration of 10^{-1}g/l of the heterosidic fraction has completely reverse the vasorelaxant effect induced by the concentration of 10^{-2}g/l.

To determine whether the concentration of 10^{-1}g/l of the heterosidic fraction tested alone induces a vasoconstriction effect, we had tested it on intact and denuded rings of thoracic aorta pre-contracted by PHE (Fig. 3 C1 and C2). The obtained results showed that 10^{-1}g/l of the heterosidic fraction has induced two distinct effects, a vasodilator effect (Rmax= 76 ±2%) followed by a vasoconstriction effect of the same amplitude. These effects disappeared on denuded ring, confirming that these two effects were endothelium-dependents. This result suggests that the heterosidic fraction contains two types of active principles, vasoconstrictors and vasodilators compounds.

Our previous study showed that the aqueous extract of *A. unedo* leaves provoked an endothelium-dependent-vasodilator effect mediated by NO/cGMP pathway (Ziyyat et al. 2002). Therefore, in order to understand whether the vasoconstriction effect of the heterosidic fraction depends on its vasorelaxant action, we pre-incubated the aortic rings in the presence of N^G nitro-L-arginine methyl ester (L-NAME) 100µM for 20min to block the nitric oxide synthesis and thereby the vasodilator effect. Then we tested directly the heterosidic fraction at 10^{-1}g/l from the basic tension and/or in rings pre-contracted by 1 µM of PHE (Fig. 3D1 and

D2). It was noted that the vasoconstriction effect of the heterosidic fraction reappeared and potentiated the effect of PHE by 0.2 ±0.02g. The same result was obtained in the basic tension with Cmax= 0.3 ±0.1g. These results showed that the vasoconstriction effect is independent of the vasodilator effect.

3.1.3. Effect of the aqueous fraction on response of aorta rings

Cumulative addition of the concentrations ranging from 10^{-4} to 10^{-1}g/l of the aqueous fraction induced concentration-dependent relaxation of the PHE induced contractile response (Fig. 4). The maximum relaxation was obtained at the concentration of 10^{-2}g/l, expressed as a percentage of the maximal phenylephrine-induced contraction is about Rmax=77 ± 3% (n =7). This vasodilator effect was inhibited by removal of the endothelium suggesting that this effect is endothelium dependent.

3.1.4. Polyphenolic and flavonoid contents in Arbutus unedo leaves

Table 2 shows the total polyphenols and flavonoids contents of A. unedo leaves. The total polyphenols contents were 136 ±2; 933 ±15 and 2337 ±67mg/100g of dried plant material respectively in the genin, heterosidic and aqueous fractions while the contents of flavonoids in the same fractions were respectively 85 ±2, 318 ±11 and 1370 ±55mg/100g of dry plant material. The aqueous fraction presented the highest content in total polyphenolic and flavonoid.

3.1.5. Thin layer chromatography

The main objective of this preliminary separation technique was to characterize the two fractions enriched in flavonoïds (heterosids and genin), to validate the protocol used for extraction of flavonoids and to determinate the number of compounds that exist in those fractions.

Figure 5 shows the simplified diagram of the chromatographic analysis (TLC) with the Rf calculated for the various spots appeared in both flavonoids enriched fractions. For the genin fraction, eight spots were separated with the elution solvent benzene/methanol/acetic acid (37:12:1, v/v/v). For the heterosidic enriched fraction, three spots were separated with the mixture: benzene/methanol/acetic acid (29:2:/1, v/v/v). The plates revelation by the crystallized aluminum chloride colored five spots in yellow for the genins enriched fraction and one spot among three for the heterosidic enriched fraction. The anisaldehyde showed the same results with the spots colored in green (Fig. 5). The absence of purple color for genin fraction in the presence of α-naphthol, demonstrates the absence of sugars in this phenolic

fraction. Whereas, we noted that the spot colored in yellow by aluminum chloride and in green by anisaldehyde for the heterosidic fraction, is colored in purple with α-naphthol suggesting the presence of sugars linked compounds.

Table 1: The different phases studied to separate the components of the genin fraction.

No.	mobil Phases
1	Methanol- water (50/50)
2	Acetonitrile- water (30/70)
3	Benzene- methanol- acetic acid (45/4/1)
4	Ethyl acetate- formic acid – water (40/28/42)
5	Chloroforme- methanol (50/50)
6	Toluen- Ethyl formate - formic acid (4/5/1)
7	Benzene- methanol- acetic acid (45/10/1)
8	Benzene- methanol- acetic acid (45/8/1)
9	Benzene- methanol- acetic acid (40/15/1)
10	Benzene- methanol- acetic acid (37/12/1)

Table 2: Average content of flavonoids and polyphenols in various fractions obtained from *Arbutus unedo* leaves (Test repeated 4 times).

	Genin fraction	Heterosidic fraction	Aqueous fraction
Total polyphenols (mg/100g of dried plant material)	136 ±2	933 ±15	2337 ±67
Flavonoids (mg/100g of dried plant material)	85 ±2	318 ±11	1370 ±55

3.2. Discussion

Our previous studies have shown that the aqueous extract of *A. unedo* leaves and roots delays the development of hypertension via several pathways, one of which is the improvement of vascular function (Afkir et al. 2008). In addition, we have found that the *A. unedo* roots and leaves aqueous extract, induces endothelium-dependent vasodilator effect mediated via the activation of the NO/cGMP pathway. This effect was partly attributed to tannins among them oligomeric tannins and catechin gallate (Ziyyat et al. 2002; Legssyer et al. 2004). We decided to continue this work, focusing our interest on flavonoids which are also well known in the literature by their vasodilator effects (Xu et al. 2007). In the present study, we have tested the

flavonoids extracted from *A. unedo* leaves on the rat isolated thoracic aorta, in order to explore their vascular effects. The extraction protocol allowed us to obtain two flavonoids-enriched fractions, which are free flavonoids (genin) and heterosidic flavonoids and a final aqueous fraction. The results obtained on isolated thoracic aorta with functional endothelium showed that the aqueous fraction induced a potent vasodilator effect concentration-dependent with a maximum relaxation Rmax=77 ±3%. However, this effect disappeared when the aorta rings were denuded (endothelium destroyed), suggesting its dependency of the endothelium. This result is similar to that obtained with the total extract of *A. unedo* leaves (Ziyyat et al. 2002). On the other hand, the free flavonoids (genins) induced a moderate vasoconstriction effect that does not appear on denuded ring suggesting also that this effect is endothelium-dependent.

Furthermore, the heterosidic fraction produces two distinct effects, a potent vasodilator effect at the concentration of 10^{-2}g/l with a maximum relaxation Rmax= 77 ±2%, whereas it induces a vasoconstriction effect at the concentration of 10^{-1}g/l at the same amplitude of the vasodilator effect obtained by 10^{-2}g/l. This result suggests that this fraction contains two types of compounds with opposite effects: the vasodilators and others vasoconstrictors. When we tested directly the concentration of 10^{-1}g/l on a ring pre-contracted at 1µM of PHE, it produces also a double effect; it relaxed firstly the muscle in the same manner as the effect obtained at the dose of 10^{-2}g/l with a maximum of relaxation Rmax= 76 ±2% and then it contracted it until plateau suggesting that the vasoconstrictor effect obtained at 10^{-1}g/l of heterosidic fraction occurs after the vasodilator effect.

To verify whether the vasoconstrictor effect obtained at 10^{-1}g/l of the heterosidic fraction is related or not to the vasorelaxant one, the aortic rings were incubated in presence of 100µM of L-NAME (NO synthesis inhibitor) for 20min in order to block the vasodilator effect. The obtained results showed that this concentration tested on PHE pre-contracted rings (1µM) potentiate the contractile response to PHE by 0.2 ±0.02g in the absence of the vasorelaxant effect. On the basic tension, this concentration produce a moderate vasoconstriction effect (Cmax= 0.3 ±0.1g). This result suggests that the vasoconstriction effect obtained is independent of the pathway of NO synthesis, and then independent of the vasodilator effect.

Moreover, the quantification of total polyphenols and flavonoids contents in plant sample showed there abundance in *A. unedo* leaves. So the vasodilatation effects of *A. unedo* leaves could be attributed to the presence of these valuable compounds. In fact, several studies have shown that the genins compounds such as the kaempferol, quercetin and its derivatives and

luteolin that are present in *A. unedo* produce a very potent vasorelaxation dependent or not from the endothelium (Xu et al. 2007; Dong et al. 2009; Qian et al. 2010; Chirumbolo 2012; Kukongviriyapan et al. 2012). In contrast, the genin enriched fraction of *A. unedo* induced a moderate vasoconstriction effect which could be due either to the presence of vasoconstrictors compounds whose effect are more powerful than the vasorelaxant compounds or in the presence of the compounds with a double effect depending on the concentration. For example, catechin derivatives that were detected in *A. unedo* (Dumon et al. 1996) and gallic acid potentiate the contractile response of PHE at low dose and induce a vasodilator effect in high doses (Andriambeloson et al. 1998; Sanae et al. 2002). In addition, it has been shown in the literature that the presence of sugar reduces the vasorelaxation effect obtained with the genin of the same molecule (Xu et al. 2007).

Whereas the heterosidic enriched fraction from *A. unedo* leaves produce a very potent vasorelaxation effect at the concentration of 10^{-2}g/l which could be due to the presence of such molecules in *A. unedo:* hyperoside, rutin, Afzelin; juglanin; avicularin; quercitrosid and myricetin of glucosids (Dauget & Foucher, 1982; Fiorentino et al. 2007; Males et al. 2013). For the vasorelaxant effect produced by the aqueous fraction, it may be due firstly to the presence of high polar heterosidic flavonoids and secondly to tannins who also show a vasorelaxation effects according to the literature (Romano & Lograno 2009). It is believed that further phytochemical studies will be necessary in order to isolate the active principles responsible for the vasorelaxant activity.

Since flavonoids are widespread in our plant foods especially fruits, it would be very useful to know their bioavailability and therefore their ability to act on the blood vessels in either the right (vasodilation) or in the wrong way (vasoconstriction). The vasodilator effect induced by heterosids fraction (at low dose), and by the aqueous fraction confirm the protective properties of flavonoids against cardiovascular diseases. Indeed, flavonoids are well known for their antiatherogenic, antioxidant, vasodilator, antihypertensive, antiplatelet and anti-ischemic properties (for review see Middleton et al., 2000; Martin and Andriantsitohaina, 2002 ; Curin and Andriantsitohaina, 2005 ; Almeida Rezende et al, 2015). On the contrary, the vasoconstrictor effect induced by the genin fraction and by the heterosids fraction (at high dose) would be rather harmful for the organism, since such an effect, if it exists all times in vivo could lead to increased blood pressure, alteration of the vascular endothelium and can cause significant damage.

Although genins have the ability to easily cross the intestinal barrier (Ross and Kasum, 2002), their bioavailability is limited because of their low solubility in aqueous media (Hu, 2007). In addition, these molecules are rarely found free in nature since the flavonoids are often glycosylated by different sugars and esters and may also be present as polymers (Borel, 2014). So it is unlikely to see a vasoconstrictor effect due to these molecules in vivo. Similarly for heterosids, it would be difficult to achieve a sufficient concentration to induce a vasoconstrictor effect in vivo since the bioavailability of most flavonoids is very low (around 10%) (Hu, 2007).

4. Conclusions

The present finding indicates that the genins-enriched fraction showed a moderate endothelium-dependent vasoconstriction effect while the aqueous fraction induced a potent vasodilator endothelium-dependent effect. The heterosidic-enriched fraction showed a double effect, a relaxation followed by a contraction. These effects are endothelium-dependent but do not depend on one another, which suggest the presence of both constristor and vasodilator principles in this fraction. The vasorelaxant effect achieved with the aqueous and heterosidic fractions are partly attributed to flavonoids compounds. These results confirm the validity of the traditional use of *A. unedo* in hypertension treatment.

Competing interests

The authors declare that they have no competing interests.

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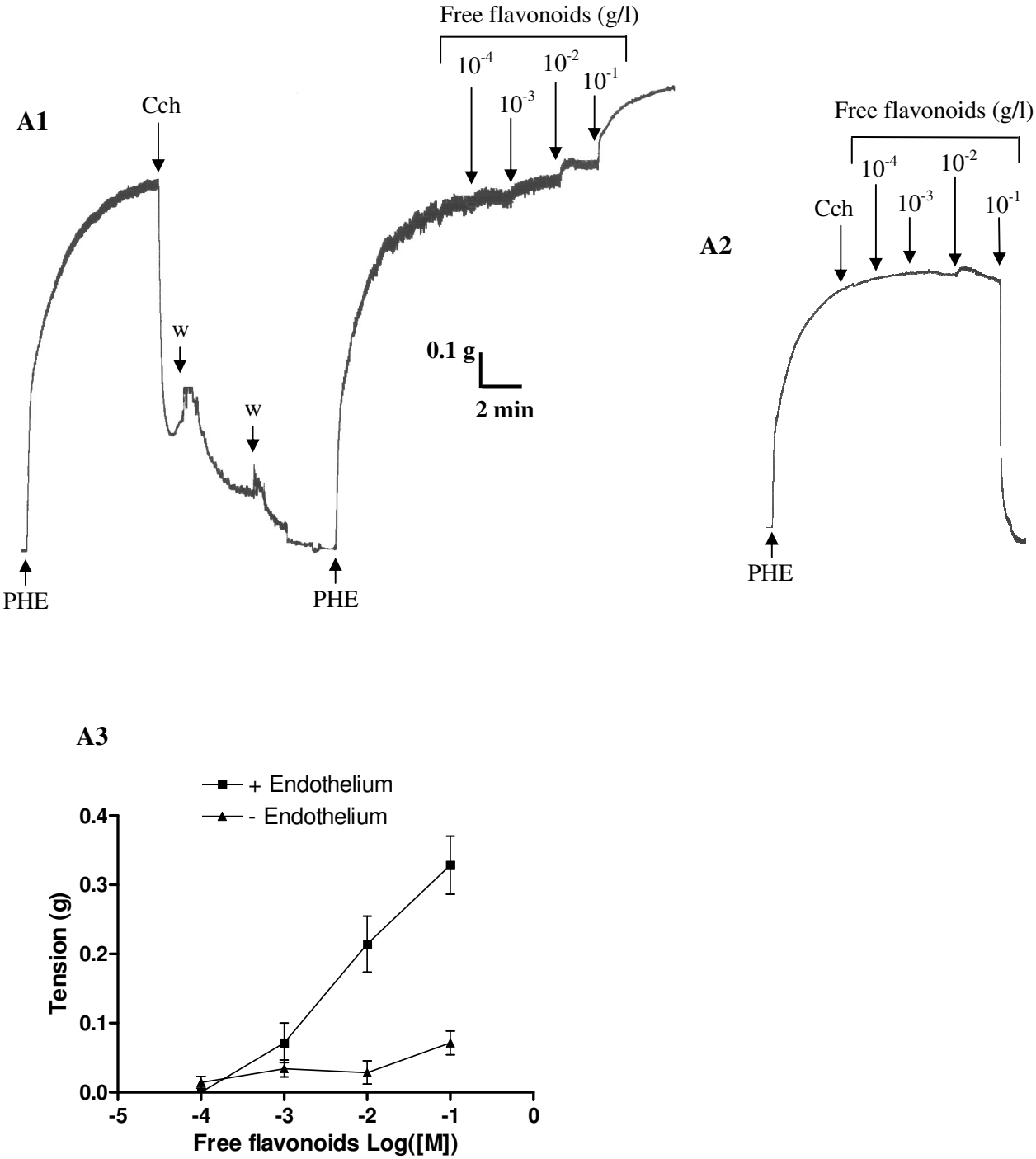

Figure 1: Original tracing showing the vasoconstriction effect produced by the genin enriched fraction (free flavonoids) from *A.unedo* leaves at cumulative concentration (10^{-4}-10^{-1}g/l) on a ring pre-contracted by phenylephrine (PHE) intact (A1) and denuded (A2). A3 shows the concentration-response curves in the presence and absence of endothelium. w: wash; Cch: Carbachol; NPS: sodium nitroprusside; PHE: Phenylephrine

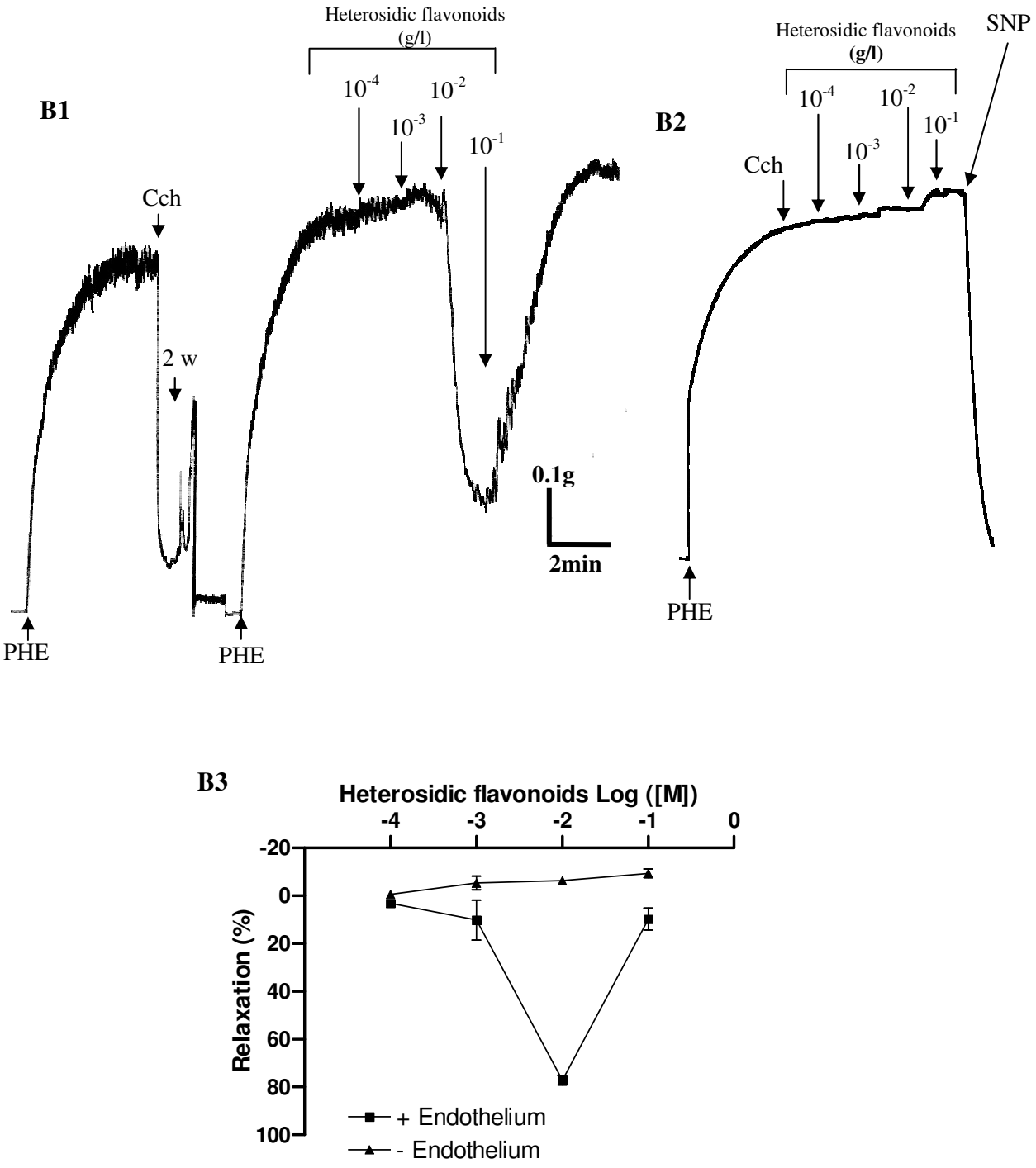

Figure 2: Original tracing showing the vascular effect of heterosidic flavonoids from *A. unedo* leaves on a ring pre-contracted aorta by PHE: (B1) intact ring and (B2) denuded ring. Concentration-response curves are shown in B3. w: wash; Cch: Carbachol; NPS: sodium nitroprusside; PHE: Phenylephrine.

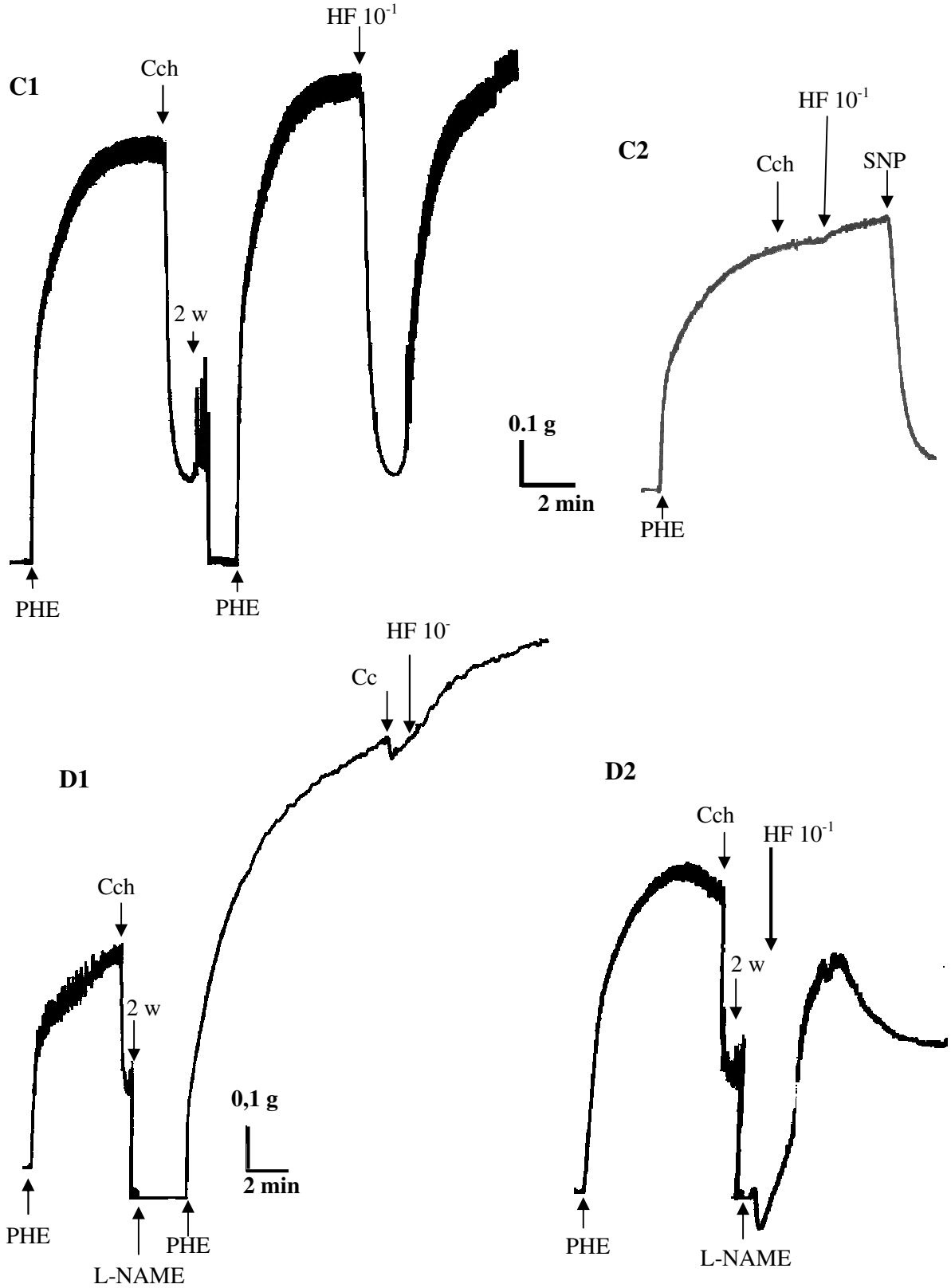

Figure 3: Original tracing of 10^{-1}g/l of the heterosidic fraction (HF) from *A. unedo* leaves tested alone on an intact (C1) and denuded rings of aorta (C2). (D1) and (D2) show the original tracing of the same concentration (10^{-1}g/l) of the heterosidic fraction on rings pre-incubated with L-NAME for 20 min (not observed time scale for this incubation period) and pre-contracted to PHE and in basis tension. w: wash; Cch: Carbachol; NPS: sodium nitroprusside; PHE: Phenylephrine

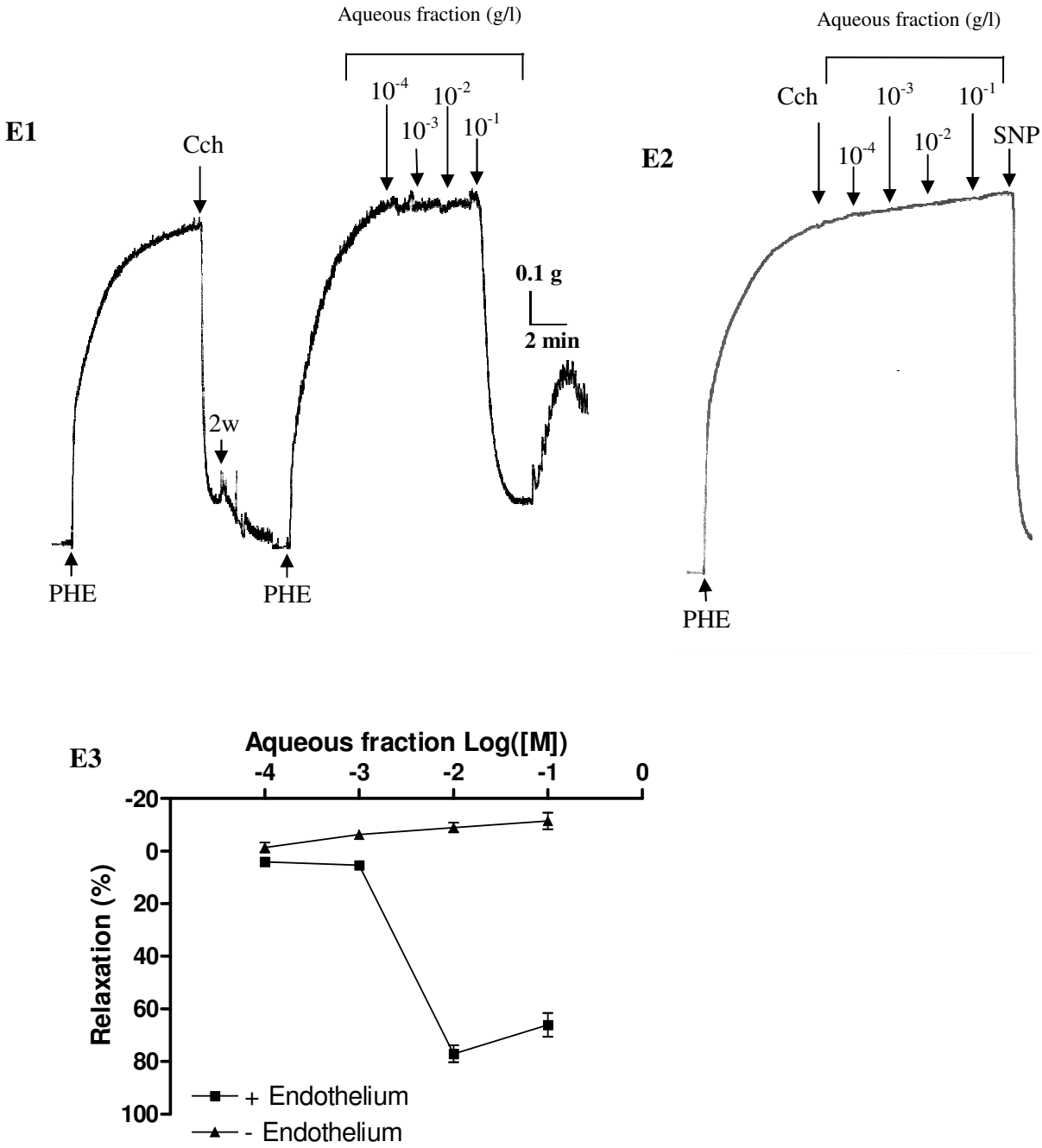

Figure 4: Original tracing showing the vascular effect of the aqueous fraction from *A.unedo* leaves on a ring of aorta pre-contracted by PHE : (E1) intact ring (E2) denuded ring. E3 shows concentration-response curves of the vascular effect of the aqueous fraction. w: wash; Cch: Carbachol; NPS: sodium nitroprusside and PHE: phenylephrine.

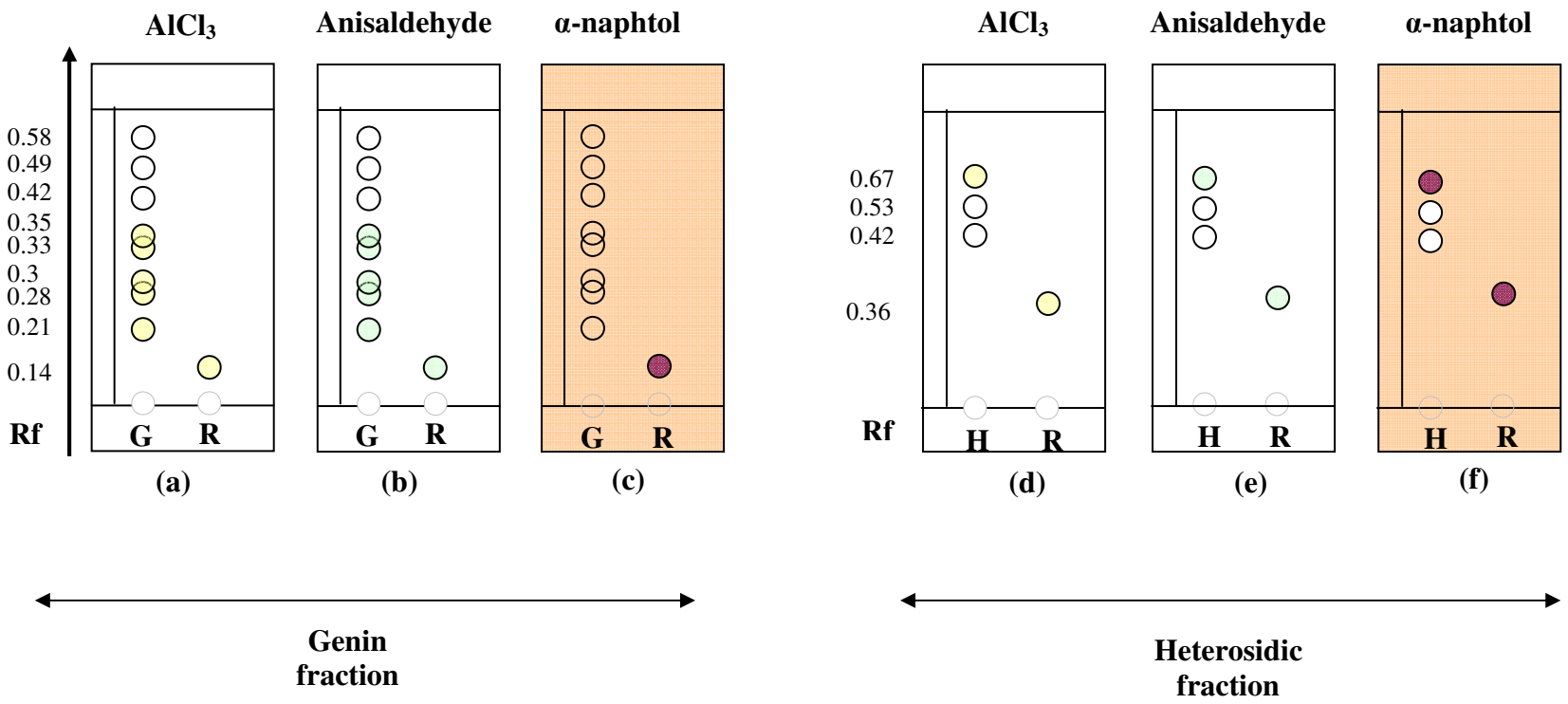

Figure 5: Simplified diagram of the chromatographic analysis (TLC) of the two fraction enriched in flavonoids (genin and heterosidic) extracted from *A.unedo* leaves. Two revealing agents are used in order to verify the presence of flavonoids : crystallized aluminum chloride (AlCl3) and anisaldehyde. The α-naphthol is used to detect the presence of heterosidic flavonoids.

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