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# Effect of simulated foregut digestion on the antioxidant capacity of plants naturally consumed by horses

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**Abstract**

Herbal medicine has impacted veterinary medicine including equine practice. Many of the postulated health-promoting effects of medicinal plants are discussed in the context of their antioxidative properties, but different factors such as digestion can have an effect on the activities of compounds. The effect of simulated foregut digestion conditions on the antioxidant capacity of seven different plants commonly consumed by horses and/or present in their complementary diet was investigated. Plants included willow tree (*Salix cinerea*), purple willow (*Salix purpurea*), willow regrowth (new sprouts growing at the foot of willows (*Salix cinerea*), oak tree (*Quercus* spp.), meadowsweet (*Filipendula ulmaria* spp.), hawthorn

(*Crataegus*), and blackthorn (*Prunus spinosa*). Aqueous extracts obtained from the bark and from the leaves were compared. Two methods, Oxygen Radical Absorbance Capacity (ORAC) and Trolox equivalent antioxidant capacity (TEAC) using 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays, were used to measure the total antioxidant capacity (TAC) of the extracts before and after the digestion process. The results showed that the TAC of all studied plant extracts decreased ($P< 0.05$) following the digestion process indicating that the active compounds present in the extracts were degraded. In fact, a decrease in TAC values of up to 80 % and 83 % was observed using TEAC and ORAC assays respectively. Such a drop in the TAC must be taken into account when considering the antioxidative efficiency of plants consumed by animals.

**Keywords:** TEAC Trolox equivalent antioxidant capacity using 2,2-azinobis-(3-ethylbenzothiazoline-6- sulfonic acid) (ABTS), ORAC oxygen radical absorbance capacity, herbal extract, equine.

**Abbreviations:**

AAPH, 2,2'-azobis-(2-methylpropionamidine) dihydrochloride

ABTS, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)

ASE, accelerated solvent extraction system

DMSO, dimethyl sulfoxide

HCl, chloridic acid

KCl, potassium chloride

$K_2S_2O_8$, potassium persulfate

$KH_2PO_4$, potassium phosphate

$Na_2HPO_4.2H_2O$, di-sodium hydrogen phosphate dihydrate

NaCl, sodium chloride

NaOH, sodium hydroxide

ORAC, oxygen radical absorbance capacity

PBS, phosphate buffer saline

TAC, total antioxidant capacity

TEAC, trolox equivalent antioxidant capacity

## 1. Introduction

Plant–based recipes have been traditionally used for their potential health promoting properties since ancient times, but they have gained increased interest over the past two decades. As a consequence, the global phytotherapy and homeopathy market value has increased from $19.4 billion in 1999 to $83 billion in 2008 (Robinson and Zhang, 2011). The use of medicinal plants is impacting not only human but also veterinary medicine, with herbal treatments being increasingly used for animal performance improvement or as alternative therapies for injuries or diseases. It is a traditional practice for horse owners, breeders or trainers to use various plant extracts for therapeutic applications or feed additives (Elghandour et al., 2018). With regard to equine diet, willow (*Salix*) bark is used as feed supplement to treat joint and metabolic problems, and ginseng, which is one of the most commonly used supplements, is used to reduce stress, improve performance and stimulate the immune system (Williams and Lamprecht, 2008). However some plant species such as St. John's wort (*Hypericum perforatum*), mistle-toe (*Phoradendron flavescens*), black cherry (*Prunus serotine*), and castor bean (*Ricinus communis*) are considered to be toxic for equines (Lans et al., 2006).

The health-promoting effects of plant extracts are often attributed to their antioxidative properties. These extracts are in fact known to possess potent antioxidant properties mainly

due to the presence of phenolic compounds. Such antioxidant compounds have been identified in plant extracts used as feed additives in horse nutrition (Elghandour et al., 2018; Williams and Lamprecht, 2008). Diet supplementation with such compounds can reduce the risk of developing diseases related to oxidative stress. Siard et al. suggested that polyphenols can be used to combat chronic inflammation in horses (Siard et al., 2016). In fact, they are known to quench the proliferation of radical oxygen and reactive nitrogen species which are implicated in etiology of different diseases such as cancer, obesity, coronary heart diseases, diabetes, asthma and rhinitis in humans (Barnham et al., 2004; Eberhardt et al., 2000; Finkel and Holbrook, 2000).

Chemical and biochemical assays exist that can be used to quantify the Total Antioxidant Capacity (TAC) of plant extracts. These assays involve reactions with either a single electron transfer or a hydrogen atom transfer (Huang et al., 2005). Yet, a major drawback of these assays is the lack of information on the bioavailability of the bioactive molecules following the digestion process for instance (Stahl et al., 2002). *In vitro* studies have shown that some individual antioxidants may remain stable and active throughout the human digestion (Ryan et al., 2008; Wootton-Beard et al., 2011). Such data is lacking when it comes to the equine digestion system which is quite different from the human one.

The aim of this study was to evaluate the stability of the antioxidant capacity of different plants commonly consumed by horses and/or used in their complementary diet after a simulated *in vitro* horse digestion. The TAC was evaluated using the oxygen radical absorbance capacity (ORAC) and Trolox equivalent antioxidant capacity (TEAC) using 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays.

## 2. Materials and methods

### 2.1 Reagents

All solvents used in this study were of analytical grade. Ethanol, dimethyl sulfoxide (DMSO), 2,2'-azino-bis-(3-ethylbenthioazoline-6-sulfonic acid) (ABTS), 3H-xanthene-3-one (fluorescein), 2,2'-azobis-(2-methylpropionamidine) dihydrochloride (AAPH), and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Steinheim, Germany). Chloridic acid (HCl), sodium hydroxide (NaOH) and sodium chloride (NaCl) were obtained from Thermo Fisher Scientific (Illkirch-Graffenstaden, France). Porcine pepsin (500 U/g USP) was purchased from VWR (Fontenay-sous-bois, France). Porcine pancreatin (25 000 U/g USP), potassium persulfate ($K_2S_2O_8$) and potassium chloride (KCl) were obtained from Alfa Aesar (Kandel, Germany). Di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$) and potassium phosphate ($KH_2PO_4$) were purchased from Merck (Molsheim, France). Milli-Q water (18.2 MΩ) was generated by Millipore synergy system (Molsheim, France).

### 2.2 Plants

Plants were selected based on their spontaneous consumption by semi-free ranging domestic horses (group 1, n = 13, pasture = 17ha, Louhans, France; group 2, n = 7, pasture = 10ha, Hauteville-Lompnes, France). Plant consumption was based on behavioral observation conducted 6 hours per day, 6 days a week over a period of 3 months. Behavioral observations were made by two observers on foot that maintained a minimum distance of 50 meters with horses. Observations were recorded using a videocamera and/or a voice recorder. Observers recorded any feeding event that was not grass grazing by stating the start and end time as well as the number of bites. In the case of tree browsing, observers counted the number of branches that were eaten while also indicating their approximate length and whether leaves

were present. Plant species identification was first made at a distance and was later confirmed by the observer once the feeding event was over and the focal horse had moved away.

During the period of observation, horses did not receive any supplemental hay. Nearby rivers provided an *ad libitum* access to fresh water. In total the leaves and barks of 7 different plants were manually collected: willow tree (*Salix cinerea*), purple willow (*Salix purpurea*), willow regrowth (new sprouts growing at the foot of willows), oak tree (*Quercus* spp.), meadowsweet (*Filipendula ulmaria*), hawthorn (*Crataegus* spp.) and blackthorn (*Prunus spinosa*). For each plant species, samples were taken from 7 different specimens that were spread all over the pasture and at least 30 meters apart. A total of 700 g of plant material was collected for each specimen (total 7 x 700 g = 4900 g for each plant species). Plant samples were stored at –20°C within 2 hours of collection and then cryogenically grounded with a grid of 1 mm in diameter in order to obtain a fine powder which was then stored at room temperature in sealed bags protected from the light.

### 2.3 Simulated foregut digestion

The simulated foregut horse digestion was adapted from Abdouli and Attia (2007). The used protocol simulated the digestion of feed in the stomach and small intestine of the horse. First, a pepsin aqueous solution at 10 g/L was prepared in 0.1 mol/L HCl at a final pH of 2.0. Then, 1 g of finely ground dry plant was added to 20 mL of the previously prepared pepsin solution. This mixture was incubated at 39 °C in a temperature-controlled oven under agitation for 2 hours. This first step simulated the digestion in the horse stomach. After the required incubation time had elapsed, 12 mL of a solution of NaOH at 0.2 mol/L was added to the previous mixture to obtain a pH of 7. Then, 13 mL of an aqueous pancreatin solution at 10 g/L were added. The resulting mixture was incubated at 39 °C under agitation for additional 4 hours. In those conditions, the digestion in the horse small intestine was simulated as

144 pancreatin was a mixture of amylase and lipase, two necessary enzymes for this digestion.
145 Finally, to neutralize the enzymes activity, the mixture was placed at 100 °C for 3 min. The
146 mixture was then placed at -80 °C overnight and lyophilized (Labconco FreeZone, Fisher
147 Scientific, Illkirch, France) for about 48 hours until a dry powder was obtained.

148

149 **2.4 *Extraction***

150 Plant extracts were obtained by solid-liquid extraction under pressure using an ASE-350
151 Accelerated Solvent Extraction system (Dionex, Sunnyvale, CA, USA). For each plant, three
152 extracts were done for the leaves and three extracts for the bark. The extraction solvent was a
153 50/50 % (v/v) mixture of water and ethanol. For the extraction, 1 g of finely grounded plant
154 powder or 1 g of plant which was subjected to the simulated foregut digestion mixed with
155 chemically inert Fontainebleau sand (previously heated at 600 °C for 4 hours) were placed
156 into a 10-mL stainless steel extraction cell. Two 27 mm cellulose filters (Dionex, Sunnyvale,
157 CA, USA) were placed at the bottom and at the top of the extraction cell. The extraction cell
158 was then subjected to three cycles of extraction at 40 °C and 100 bars. The duration of one
159 cycle was 10 min. The whole extraction process took 30 min. After extraction, the solvent
160 was evaporated under a gentle nitrogen flow. The extracts were weighed and kept at 4 °C in
161 amber vials to protect the molecules from light degradation.
162 To evaluate the antioxidant capacity, the extracts were dissolved at a concentration of
163 1 mg/mL in DMSO prior to the different assays carried out.

164

165 **2.5 *Antioxidant capacity***

For both TEAC and ORAC assays, trolox was used as a standard for quantitative assessment of the antioxidant activity and calibration curves were done using a range of concentration from 50 to 500 µmol/L and were prepared in Milli-Q water (Molsheim, France)..

### *2.5.1 TEAC*

The principle of the antioxidant capacity evaluation by the TEAC assay is based on the electron loss of the cationic radical $ABTS^{\cdot+}$. Indeed, during this reduction by an antioxidant, $ABTS^{\cdot+}$, initially colored blue-green, becomes colorless. The measured absorbance can then be directly and quantitatively linked to the total antioxidant capacity of the extracts. The radical cation $ABTS^{\cdot+}$ was formed by adding a solution of potassium persulfate at 2.5 mmol/L to a solution of ABTS at 7.5 mmol/L. The solution was left overnight at 4 °C protected from light exposure. From this $ABTS^{\cdot+}$ stock solution, a 2 % dilution in a phosphate buffer saline (PBS) was obtained. The PBS was prepared by dissolving 8.2 g of NaCl, 1.4 g of $Na_2HPO_4$, 0.27 g of $KH_2PO_4$ and 0.15 g of KCl in 1 L of Milli-Q water. The TEAC assay was conducted on a 96-wells microplate (Thermo Fisher scientific, Newport, United Kingdom) in which 10 µL of each extract and 200 µL of the 2 % $ABTS^{\cdot+}$ solution were added in each well. To promote redox reactions between the $ABTS^{\cdot+}$ and the molecules in the extracts, the microplate was placed at 37 °C for 10 min. Finally, the absorbance at 734 nm was read in each well by a Varioskan spectrophotometer (Thermo Fisher Scientific, Illkirch-Graffenstaden, France). Prior to the absorbance measurement, the plate was agitated for 10 seconds. The extracts with an antioxidant capacity were then identified by a decrease in intensity as the radical cation $ABTS^{\cdot+}$ would have lost its blue-green color. The TAC values of each extract were measured in triplicate.

The loss of TAC in plant extracts after simulated foregut digestion was calculated as follows: (TAC value of non-digested plant extract - TAC value of digested plant extract)/(TAC value of non-digested plant extract) and multiplied with 100 for reporting the loss in percent.

### 2.5.2 *ORAC*

The ORAC assay is based on a competition between a fluorescent probe and the antioxidant compounds present in the extract. The fluorescent probe used in this study was the 3H-xanthen-3-one, also known as fluorescein. This compound is degradable by oxidation reactions by a transfer of protons, leading to the extinction of its fluorescence. AAPH (2,2'-azobis-(2-methylpropionamidine)) was used as the initiator of the oxidation reactions as it dissociates at 27 °C by releasing peroxylic radicals. Antioxidant molecules present in the analyzed extract will absorb the free radicals generated by AAPH, the fluorescein will be protected and there will be a delay in the decrease of the fluorescence. The TAC of the extract is thus directly linked to the decreasing curve of the measured fluorescence delay. To quantify the TAC, the area under the fluorescence curve of the studied extracts is calculated.

The ORAC assay was conducted on a 96-wells dark microplate (Thermo Fisher scientific, Newport, United Kingdom), to prevent the degradation of the fluorescein by light. In each well, 10 µL of the extract and 150 µL of a solution of fluorescein at $2.8 \times 10^{-5}$ g/L in Milli-Q water were added. The microplate was then placed in a fluorescence microplate reader (Varioskan, Thermo Fisher Scientific, Illkirch-Graffenstaden, France), shaken for 20 sec and incubated at 37 °C for 15 min. Then, using an automatic dispenser integrated into the fluorescence reader, 30 µL of an AAPH solution in PBS at 41.5 g/L were added to each well. The microplate was agitated again for 5 sec. The fluorescence kinetics was then measured every 5 min for 2 hours with excitation and emission wavelengths of 485 nm and 530 nm respectively. The experiments for each extract were conducted in triplicate.

The percentage loss of TAC of extract plant non digested and digested was calculated as TEAC assay.

### 2.6. Statistical analysis

The means of triplicate TAC values (experimental unit) for each plant extract obtained with TEAC or ORAC assays were compared before and after simulated digestion, the paired, two-tailed *t* test was used using the software Minitab (2017) v.17. Means with P value < 0.05 were considered statistically different.

## 3. Results

In this study, two methods, TEAC and ORAC, were used to evaluate the TAC of seven selected plant extracts before and after a simulated foregut digestion. The concerned plants were willow tree, oak tree, purple willow, willow regrowth, meadowsweet, hawthorn, and blackthorn. Aqueous extracts from the leaves and from the bark of willow tree, oak tree, purple willow and willow regrowth were used. For the three remaining plants, only aqueous leaf extracts were used, either because they don't have a bark (meadowsweet) or because their bark is not consumed by horses due to the presence of large thorns (hawthorn and blackthorn).

Figure 1 shows the TAC expressed in µmol Trolox equivalent per gram of plant (µmol TE/g of plant) of each plant extract before and after simulated foregut digestion for TEAC (figure 1.A) and ORAC (figure 1.B) methods.

### 3.1. TEAC assay

Prior to simulated foregut digestion, the TAC of all plant extracts assessed using TEAC assay showed a level of antioxidant activity with values ranging from 1 538 to 10 119 µmol TE/g of plant extract. The lowest TAC value was obtained with the extract of purple willow leaves and the highest with the extract of oak tree bark.

Among bark extracts, the lowest TAC value was observed with the extract of purple willow (3 778 ± 387 µmol TE/g of plant) while the highest value was obtained with the oak tree extract (10 119 ± 1748 µmol TE/g of plant). Among leaf extracts, purple willow extract (1 538 ± 65 µmol TE/g of plant) and oak tree extract (7 083 ± 1 256 µmol TE/g of plant) showed the lowest and the highest TAC value using TEAC assay, respectively. The bark and leaf extracts of oak tree showed the highest TAC values compared to the other plant extracts. The bark extracts of willow tree, purple willow, willow regrowth and oak tree showed higher TAC values compared to their respective leaf extracts ($P < 0.05$). This suggests that the bark may contain more antioxidants than the leaves.

After the simulated foregut digestion, the TAC values of all plant extracts decreased ($P < 0.05$) (figure 1). The highest TAC value was observed with the bark extract of the willow tree (2 421 ± 322 µmol TE/g of plant) and the lowest value with blackthorn leaf extract (1 008 ± 87 µmol TE/g of plant). Table 1 shows the percentage loss of TAC before and after digestion for each plant extract for both TEAC and ORAC assays. The loss ranged from 80 % to 16 % with the TEAC assay. The TAC values of the bark and leaf extracts of oak tree, which were the highest before digestion, decreased by 80 % after being subjected to the simulated foregut digestion process (table 1). Moreover, the observed pattern with non-digested extracts of same plant, where the TAC values of bark were higher than those of leaves, was not observed with the digested extracts. In fact, the TAC values of bark extracts were similar or lower than those obtained with leaf extracts.

### 3.2. ORAC assay

For the ORAC assay, the TAC values obtained prior to simulated foregut digestion ranged from 2 216 to 16 915 µmol TE/g of plant.

Among the studied extracts, the bark extract of the willow tree showed the highest TAC value (16 915 ± 1 384 µmol TE/g of plant) while its leaf extract showed the lowest value (2 216 ± 311 µmol TE/g of plant). Among bark extracts, the lowest TAC value was observed with the extract of purple willow (3 790 ± 74 µmol TE/g of plant) while the highest value was obtained with the willow tree extract (16 915 ± 1 384 µmol TE/g of plant). Among leaf extracts, the lowest TAC value was observed with the willow tree extract (2 216 ± 311 µmol TE/g of plant) and the highest value with the oak tree extract (12 426 ± 418 µmol TE/g of plant). As with the TEAC assay, the bark extracts of the willow tree, purple willow and willow regrowth showed higher TAC values than the respective leaf extract ($P < 0.05$) (figure 1). However, this pattern was not observed with the oak tree extracts with the leaves showing higher ORAC TAC values than the bark ($P < 0.05$) (figure 1).

After the simulated foregut digestion, the ORAC TAC values of all plant extracts were lower ($P < 0.05$) and ranged from 932 to 5108 µmol TE/g of plant (figure 1). According to table 1, the decrease ranged from 83 % to 20 %. The bark extract of the willow tree which showed the highest TAC value was reduced by 83 % following simulated digestion. As with the TEAC assay, this reduction resulted in TAC values equivalent to those of leaf extracts.

## Discussion

In this study, TAC of seven different plants naturally consumed by horses was measured by two different methods, TEAC and ORAC. The TEAC method measures the capacity of the compound to donate electrons, while the ORAC method measures the capacity of a molecule to transfer hydrogen atoms. The obtained TAC values with the two methods can therefore not be compared as they represent different antioxidant phenomena measured in different conditions (Schaich et al., 2015). The TAC values were between 1 538 and 10 119 µmol

TE/g of plant using TEAC assays. By comparison, according to Jiménez-Zamora et al. (2016), the aqueous extract of green tea leaves has a TAC of about 2 289 µmol TE/g of plant as measured by the TEAC assay. This shows that the plants studied have a high antioxidant capacity as their TAC are higher or at least close to that of green tea leaves, a plant well-known for its antioxidant activity (Jiménez-Zamora et al., 2016). Furthermore, the TAC values measured for leaves were lower than those of the bark ($P < 0.05$) (figure 1). This result shows the importance of studying different parts of the plants as differences may exist in the contents of active compounds.

The effect of simulated foregut digestion on the TAC of the seven studied plants was also investigated. The results of both TEAC and ORAC assays showed a dramatic decrease in TAC following digestion (-16 % to -80 % with TEAC and -20 % to -83 % with ORAC) (table1). To the best of our knowledge, this study is the first to investigate the influence of our model of simulated foregut horse digestion on the TAC of plants and to demonstrate the decrease of TAC values following simulated foregut digestion. As far as comparison can be made, our results are in agreement with those of other studies using simulated *in vitro* human digestion which showed a similar trend (Bermúdez-Soto et al., 2007; Chen et al., 2013; McDougall et al., 2005). For example, Chen et al. studied the influence of *in vitro* human digestion on the TAC of tea juices measured by the TEAC assay. They showed that the simulated digestion process led to a decrease of the TAC values of six tea juices out of nine (Chen et al., 2013). Bermúdez-Soto et al. (2007) showed a decrease in TAC after an *in vitro* pancreatic digestion of different chemical compounds found in chokeberry juice such as anthocyanins (-43 %), flavonols (-26 %) and flavan-3-ols (-19 %). In other studies based on the TEAC assay, it was observed on the other hand that some extracts can stay stable throughout the digestive process (gastric and pancreatic) such as extract Perennial chamomile

(*Chamaemelum nobile*) or Japanese plum (*Prunus salicina)* seeds extract (Chen et al., 2015, 2016; Wootton-Beard et al., 2011).

The decrease of the TAC of the plant extracts may be due to the degradation of antioxidant compounds present in the extracts. In fact, some compounds known for their antioxidant activity, such as polyphenols – a major family of natural products found in plants- may be decomposed into different structural forms with different chemical and biological properties during the pancreatic digestion due to their sensitivity to pH variations (Bermúdez-Soto et al., 2007). Indeed it has been shown *in vitro* that the pH influences oxidoreductions of phenolic compounds. Some phenolic compounds have for instance displayed prooxidant activities at pH 7.4, while at lower pH (5.8) they were reported to possess antioxidant activities (Decker, 2009; Moran et al., 1997). Other factors such as the interactions of phenolic compounds with other food components released during digestion (iron, other minerals, dietary fiber, proteins) may influence the antioxidant properties of phenolic compounds. In fact, it has been shown that the antioxidant activity of free phenols is higher compared to iron-phenol chelates (Argyri et al., 2006; Hajji et al., 2006; Seraglio et al., 2017). In addition, the antioxidant activity of phenolic compounds also depends on their chemical structure, mainly the number and position of the hydrogen-donating hydroxyl groups on the aromatic rings (Rice-Evans et al., 1996). Previous works have also showed the degradation of anthocyanins, known antioxidant compounds, after being subjected to the alkaline conditions of the pancreatic digestion process (McDougall et al., 2005; Pérez-Vicente et al., 2002).

In this study, a simulated foregut digestion with porcine enzymes was conducted. However, the results obtained with our mimetic horse foregut digestion have to be nuanced. Firstly, the conditions of the simulated foregut horse digestion, based on the work of Abdouli and Attia (2007), used a temperature lightly higher (39°C) than equine body temperature which is in range of 37.5°C and 38°C. Secondly incubation time used in our model was

different than what happens during horse's digestion. Indeed food passes the stomach quickly (15 min) in case of it is roughage, and more slowly if it is concentrates and ingested feed reaches the caecum within 2 hours (Jones, 1993). And finally, compared to eight other species, it appear than horses pancreatic secretions into the duodenum have low enzyme activity (Frape, 2004; Lorenzo-Figueras et al., 2007). It was showed in the study of Lorenzo-Figueras et al. (2007) than the specific enzyme activity of five pancreatic enzymes of swine (amylase, lipase, elastase, trypsin, chymotrypsin) was higher than for horses. The activity measurement of five pancreatic enzymes from the adult equine and porcine pancreas was reported by Sweeney (2012), expressed as mean units per milligram of DNA: for amylase, the activity was 2.3 and 107 for equine and porcine respectively, for lipase 41.5 and 49, elastase 0.07 and 0.22, trypsin 0.13 and 0.44 and for chymotrypsin 0.36 and 2.26. Thus, considering these different points, in our study the impact of the enzymes on the TAC values is higher than expected *in vivo*. *In vivo* animal studies will have to come in support of the obtained *in vitro* data.

## Conclusion

This study shows that despites the negative impact of the digestion on their antioxidant activities, the studied plants remain a source of antioxidant compounds. Despite the acknowledged limitations of *in vitro* models that try to mimic *in vivo* conditions, they remain a useful tool to investigate enzymatic activity and its effect on biological activities of antioxidants. It would be important to investigate in detail the outcome of the bioactive compounds after the digestion by identifying their degradation products and the subsequent impact on their bioavailability and biological activity.

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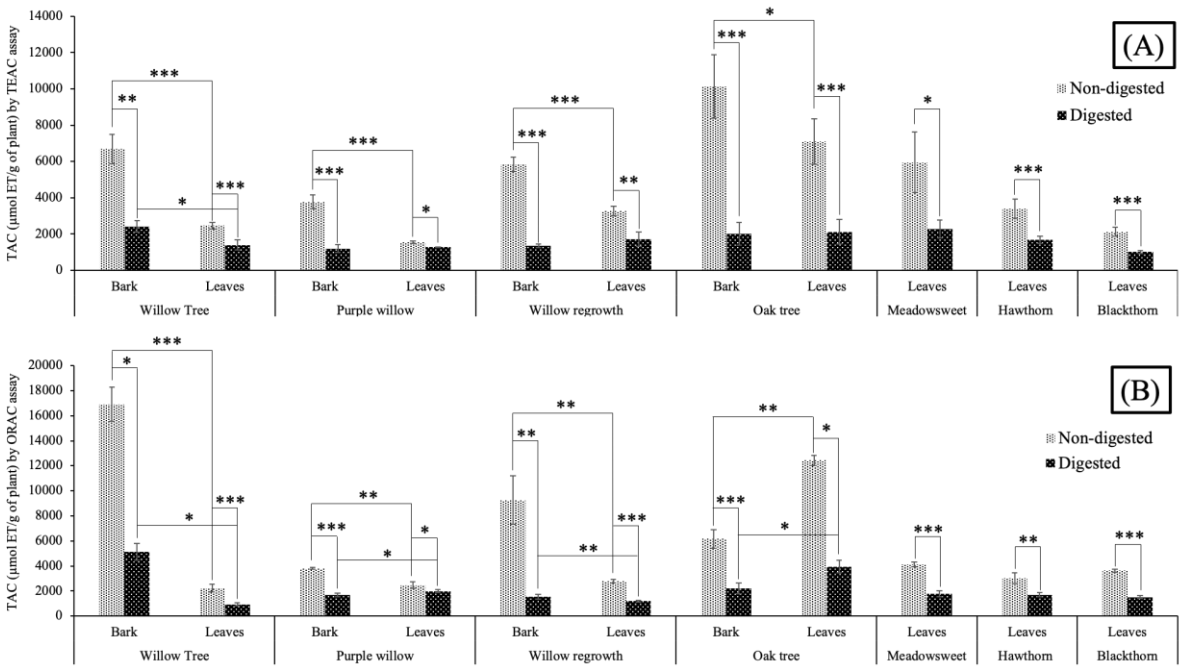


**Figure 1.** Total antioxidant capacity (TAC) of plant aqueous extracts before and after simulated foregut digestion obtained with TEAC (A) and ORAC (B) assays (n=3, *t*-test, $\alpha$=0.05, * 0.01<P<0.05; ** 0.001<P<0.01; *** P<0.001).

**Table 1**. Loss of total antioxidant capacity (TAC) in bark, leaf and whole plant extracts after simulated foregut digestion, in percent.

| **Plants** | | **TAC loss (%) TEAC assay** | **TAC loss (%) ORAC assay** |
|---|---|---|---|
| Willow Tree | Bark | 64 % | 70 % |
| | Leaves | 43 % | 58 % |
| Oak tree | Bark | 80 % | 64 % |
| | Leaves | 70 % | 68 % |
| Purple willow | Bark | 68 % | 55 % |
| | Leaves | 16 % | 20 % |
| Willow regrowth | Bark | 77 % | 83 % |
| | Leaves | 47 % | 58 % |
| Meadowsweet | Whole plant | 62 % | 57 % |
| Hawthorn | Whole plant | 51 % | 44 % |
| Blackthorn | Whole plant | 52 % | 58 % |