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Original Research Article

The effect of simulated gastro-intestinal conditions on the antioxidant activity of herbal preparations made from native Irish hawthorn

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ABSTRACT

The ability of plant phenolics to act as free radical scavengers has led to increased interest in their ability to act as antioxidants in vivo. Polyphenolic compounds, commonly present in hawthorn (*Crataegus spp.*), as well as herbal preparations of hawthorn were examined using the TEAC and DPPH assays to determine their antioxidant activity. Initial results have shown the standards to be efficient free radical scavengers. Quercetin dihydrate was found to be the most effective with ability to inhibit up to 87.9% of the DPPH radical and over 90% of the ABTS radical. The herbal preparations tested showed infusions of hawthorn leaf and flower to be almost as effective with 82.9% of DPPH radicals and 87.9% of ABTS radicals being inhibited. Most hawthorn preparations are consumed orally, however, and the effect of gastro-intestinal conditions on the ability of phenolic compounds to scavenge free radicals is not taken into account. Both, the standards and crude herbal preparations were exposed to simulated gastro-intestinal conditions to determine their effect, if any, on antioxidant activity. This study indicates that the scavenging activity of hawthorn phenolics may be reduced by the digestive process. The scavenging activity of Luteolin against ABTS radicals was found to have decreased by 67.8%. The effect of the process on the herbal preparations varied with the ability of the berry decoction to scavenge DPPH decreasing by 43% while the scavenging of the leaf and flower infusion decreased by only 1.94%.

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1. Introduction

Hawthorn has been used for centuries as a treatment for several illnesses including gout, depression, and kidney stones. The main benefit of hawthorn is believed to be its promotion of cardiovascular health (Gaby, 2006). One of the main areas of interest in regard to plant phenolics is their potential to act as antioxidants in vivo. This antioxidant activity may help prevent several disorders such as heart disease, cancer and age-related diseases (Tabart et al., 2009). Several epidemiological studies including the Zutphen study carried out in the Netherlands have found an inverse relationship between the consumption of plant phenolics and heart disease mortality (Hollman and Katan, 1997).

The main chemical constituents of interest in hawthorn are polyphenolic compounds such as flavonoids and procyanidins. The potential health benefits of these phytochemicals have been demonstrated widely in vitro but to a lesser extent in vivo. Several clinical trials, however, have been carried out to assess the cardiovascular benefits of hawthorn in vivo. With the exception of Zick et al. (2008), the studies showed that hawthorn was beneficial in the treatment of heart conditions. It was found to reduce blood pressure, especially in long-term trials greater than 3 months duration (Asgary et al., 2004), increase the capacity for exercise (Tauchert, 2002), and reduce fatigue, stress, and dyspnoea (Habs, 2004; Degenring et al., 2003). The analysis carried out by Zick, S.M. and co-workers indicated that patients who took hawthorn extract were more likely to experience heart failure progression than those who received a placebo. The study, however, was retrospective and was not originally designed to monitor heart failure progression (Zick et al., 2008).

The positive pharmacological effects of polyphenolic compounds from hawthorn on the cardiovascular system include positive effects on heart muscle contraction, vasodilatory effects resulting in lower arterial pressure and increased blood flow, improvement in oxygen utilisation, reduction of cholesterol levels, and cardioprotective effects against reduced blood flow during myocardial infarction (Furey and Tassell, 2008; Long et al., 2006; Rigelsky and Sweet, 2002; Zhang et al., 2001; Tassell et al., 2010). The majority of herbal preparations made from hawthorn are designed for oral use and, therefore, the effect of exposure to the conditions of the gastro-intestinal tract must be taken into account when examining the antioxidant activity of plant extracts. The gastro-intestinal (digestive) system is responsible for changing food into absorbable molecules by enzymes present in the different specialised compartments of the system. The stomach secretes gastric fluid which contains a hydrolytic enzyme called pepsin together with hydrochloric acid. This results in the stomach having a pH of approximately 2.0. In the small intestine the pH is slightly basic, ranging from 5.1 to 7.5, due to bile, intestinal and pancreatic juice which contains sodium bicarbonate (Mader, 1990; Vermaak et al., 2009). Pharmaceutical drugs are often protected by an enteric coating to avoid degradation of active compounds by the acidic conditions in the stomach (Liu et al., 2009). This leads to the question of the stability of bioactive compounds from hawthorn in these acidic conditions when consumed orally. It may also be prudent to design pharmaceutical formulations for herbal preparations that serve to protect the integrity of the bioactive constituents.

This study aims to examine the effect of the digestive process on the antioxidant activity of phenolic standards commonly present in hawthorn and herbal preparations. This will be achieved by exposing the standards and preparations to simulated gastro-intestinal conditions in vitro. Simulated gastric and intestinal fluid can be prepared and used rapidly and safely to indicate possible effects caused by digestion.

2. Materials and methods

2.1. Chemicals

Methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), rutin, epicatechin, chlorogenic acid, isoquercitrin, potassium persulphate, gallic acid, Folin–Ciocalteu reagent, sodium hydroxide, potassium phosphate monobasic, hydrochloric acid, and sodium carbonate anhydrous were purchased from Sigma–Aldrich (Ireland), pancreatin and pepsin were purchased from Fisher Scientific (Ireland) while quercetin and luteolin were purchased from Phytolab GmBH (Germany).

2.2. Plant material

Dried plant material (mixture of *Crataegus laevigata* or *Crataegus monogyna*; species used interchangeably in herbal medicine) was obtained from Rosari Kingston (Medical Herbalist) of West Cork Herb Farm (Cork, Ireland). The plant material was identified against a voucher specimen in the WCHF herbarium. It was stored in vacuum bags in the dark at room temperature when not in use.

2.3. Herbal preparations

2.3.1. Hawthorn leaf and flower infusion

Dried leaf and flower material (2–3 g) was placed in a tea sieve and 250 ml of boiling water was poured over the herb. The tea sieve was left standing in the water for 8 min. The sieve was then removed and the infusion was then stored at 4 °C in amber bottles (Davies, 2000)

2.3.2. Hawthorn berry decoction

Dried berries (7 g) were added to 900 ml of distilled water in a beaker. The mixture was brought to the boil and then allowed to simmer for 25 min. The berries were then removed by straining the liquid over time through a sieve and the liquid portion was placed in amber bottles and stored at 4 °C (Davies, 2000).

2.3.3. Tincture of hawthorn

56 g of plant material (one tincture made with leaf and flower and another from the berries only) were placed in a blender and 250 ml of vodka was added. Vodka (Saratov, 27.5%) was chosen due to previous reports of the antioxidant activity of whiskey (McPhail et al., 1999). As an alternative to commercial ethanol, where a licence and record keeping usage of ethanol