Sainfoin (Onobrychis viciifolia) is an excellent fodder legume with very high voluntary intakes by cattle, sheep and horses. Ruminants utilise sainfoin protein much more efficiently than lucerne or soy protein. More efficient nutrient utilisation from sainfoin leads to less emission of nitrogen, and methane, which is one of the most damaging greenhouse gases. Research also suggests that sainfoin possesses natural anthelmintic properties. It could therefore serve as an ideal fodder legume in more sustainable livestock farming systems.1

This interdisciplinary research examines the hypothesis that the condensed tannins are responsible for the above mentioned benefits of sainfoin. However, there are contradictory reports in the literature concerning the structures and molecular weights of sainfoin tannins.2 Therefore, detailed chemical analysis is required to identify its active, beneficial tannins. This structure-activity relationship study will be of use in future plant breeding/selection studies.

## Introduction

### Sainfoin

Sainfoin (Onobrychis viciifolia) is an acceptable fodder legume with high voluntary intakes by cattle, sheep and horses. Ruminants utilise sainfoin protein much more efficiently than lucerne or soy protein. More efficient nutrient utilisation from sainfoin leads to less emission of nitrogen, and methane, which is one of the most damaging greenhouse gases. Research also suggests that sainfoin possesses natural anthelmintic properties. It could therefore serve as an ideal fodder legume in more sustainable livestock farming systems.1

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## Tannin extraction & fractionation

**Procedure**

1. Freeze dry Onobrychis viciifolia var. Cotswold Common (50% flowering, whole plants)
2. Extract with acetone/H2O-7:3
3. Separate into 2 fractions (with Sephadex LH20) resp. 9 fractions (with Toyopearl HW50F; first 3 fractions were eluted with water and were not analysed)

<table>
<thead>
<tr>
<th>Column</th>
<th>Packing</th>
<th>MeOH/H2O-1:1 (A)</th>
<th>acetone/H2O-7:3 (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sephadex LH20</td>
<td>13.1%</td>
<td>4.1%</td>
<td></td>
</tr>
<tr>
<td>Toyopearl HW50F</td>
<td>0.9% (A1)</td>
<td>0.1% (B1) 1.2% (B3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.5% (A2)</td>
<td>2.3% (B2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.6% (A3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0% (B3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

% is expressed avg extract/100g sainfoin (on dry matter basis)
* may contain some sugars

### Table 1

## Isothermal titration calorimetry

### Binding affinity

<table>
<thead>
<tr>
<th>Sumac tannins (GT)</th>
<th>Larra tannins (GT)</th>
<th>Sainfoin tannins (CT)</th>
<th>Chestnut tannins (ET)</th>
<th>Myrabolan tannins (ET)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ks (M^-1)</td>
<td>1.7x10^4</td>
<td>1.0x10^4</td>
<td>6.6x10^4</td>
<td>9.0x10^6</td>
</tr>
</tbody>
</table>

ΔH_m (kJ/mol) = -30.3 -33.0 -21.82 -39.8 -58.1
ΔS_m (kJ/mol) = -0.45 -10.17 0.03 -22.94 -41.87
ΔG_m (kJ/mol) = -29.85 -22.83 -21.79 -16.86 -16.23

### Stability of tannin-BSA complex

| N | 9.4 | 2.5 | 35.2 | 17.7 | 22.0 |

### Table 2

**Experimental procedure**

A solution of 3mg/ml of sainfoin was titrated into a 10 μl BSA solution in 50 mM citric acid buffer (pH 6) at 298K.

## Conclusion

1. Calorimetry shows that both the binding affinity and the stability of the sainfoin tannin-BSA complex falls between gallotannins and ellagittannins.
2. Toyopearl enabled the isolation of a fraction containing high molecular weight tannins.
3. The high molecular weight fraction has a higher prodelphinidin content, this confirms earlier studies.
4. Sainfoin contains a highly complex mixture of condensed tannins in terms of PC:PD ratios and molecular weights. Comparisons between the tannins from sainfoin, Lotus pedunculatus and Lotus corniculatus are ongoing in order to determine if this complexity is the key to their beneficial effects of sainfoin.

## References & acknowledgement


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