Novel Food Assessment of Tinofend

Tinofend is a dried water extract of the herb *Tinospora cordifolia*

The herb is listed in the EU Novel Food catalogue.

Tinospora cordifolia

Description
Tinospora cordifolia is a large, climbing shrub. A deciduous unknown that grows to 1.0 meters (3.3 feet) high by 0.5 meters (1.65 feet) wide and prefers many types of soil ranging from acid to alkaline and partial to full sun with moderate moisture. This plant has hermaphrodite flowers. "Guduchi" tea from *Tinospora cordifolia* was on the market in the EU to a significant degree.
Novel Food Status

Teas from the herb are therefore not Novel. It does not mention which part of the plant is used in the tea. However tea products currently on the market in the EU are from the Stem. (See link below to an example)

https://www.satnam.de/en/guduchi-balance-tea-organic-20-teabags.html

As Tinofend is a dried water extract of the stem of *Tinospora cordifolia one* would expect it to have a similar chemical profile as for a tea made with the herb because a tea is essentially an undried water extract.

The question how similar is the chemical profile likely to be? will depend on the similarities and differences in the extraction process used to make the Tinofend compared to that in making a tea.

The extraction process for Tinofend uses 625 Kgs of dried herb of size 0.75 to 1.5 cm which is added to 3000 Litters of water at 85 to 90 ^{o}C. It is held at that temperature for 3 hours before being filtered. It is not stirred.

Typically for a *Tinospora cordifolia* herb tea 240 ml of boiling water is added to 2 g of the herb (size 1 - 2 mm) and allowed to brew for 10 minutes.

The main differences between the 2 processes are:

1. The temperature which is 100 ^{o}C for the tea and 80-90 ^{o}C for the Tinofend
2. The time of contact between the herb and the water, 3 hours for Tinofend and 10 minutes for the tea
3. The ratio of water to herb which is 4.8 for the Tinofned and 120 for the Tea.
4. The surface area per unit weight of the herb is much higher for the tea extraction

The main similarities are:

1. The herb is the same

2. The solvent is the same (water)
3. The solubility's of the components of the herb in water will be the same as these are based on Thermodynamic factors.

Can we compare the effect that the differences in the processes are likely to have on the chemical profile of the extracted components?

Unlike solubility, dissolution is a Kinetic process and the rate of dissolution of a solute into a solvent can be calculated using the Noyes- Whitney equation:

$dm/dt = AD (Cs-Sp)/d$

Where dm/dt is the rate of dissolution

A is the surface area of the solute

D is the diffusion coefficient

m is the mass

t is the time

d is the thickness of the concentration gradient

Cs is the solute surface saturation concentration

Cp is the concentration of the solute in the bulk solution

The Diffusion Coefficient is separately defined by the Stokes Einstein Equation

$D = KT/6\pi\eta r$

Where

K is the Bolztman constant

η is the viscosity of the solvent

π is 3.14159

T is the absolute temperature

r is the radius of the solute

Looking at both the Noyes-Whitney and the Stokes-Einstein equation we can see the factors which will influence the dissolution rate.

Some are intuitive. As the temperature is increased the rate of dissolution increases because of the effect of Temperature on the diffusion coefficient. It will act in 2 ways. Firstly by increasing the value of T in the Stokes-Einstein equation and secondly by decreasing the viscosity η in the same equation. Both these changes will increase the Diffusion Coefficient and thus also the rate of dissolution. In the actual case if we consider the tea to be extracted at 100 ^{o}C and the Tinofend at 90 ^{o}C then the higher temperature used to extract the Tea and the lower viscosity of the water at 100 ^{o}C (0.2825×10^{-3} PaS) compared to 90^{o}C (0.314×10^{-3} PaS) will lead to a 15% decrease in the rate of dissolution of the Tinofend compared to the tea.

The surface area of solute will affect the dissolution rate in 2 ways. An increase in surface area will lead to a higher dissolution rate directly by increasing the value of A in the Noyes-Whitney equation. Also the Diffusion coefficient will increase as a result of the reduction in the solute radius, leading to an increase in the rate of dissolution. In the manufacture of the Tinofned the raw material is cut to between 0.75 to 1.5 cm which is large compared to a commercial tea where the size would be around 1 to 2 mm. Assuming the size of the Tinofend raw material to be 0.75 cm and that of the Tea to be 2 mm it will lead to a surface area per unit weight of the Tinofend to be about 3.75 times lower than the tea and the Diffusion Coefficient also 3.75 times lower. This means that the effect of the lower surface area of the Tinofend starting material leads to a lower rate of dissolution of about 14 times compared to the tea.

The concentration term (Cs-Cp) in the Noyes-Whitney equation has an effect on the dissolution rate and this will dependant on the amount of water being used. The higher the value of Cp then the slower the rate of dissolution. In the Tinofend extraction there is 25 times less water per unit weight of herb compared to the Tea extraction and thus a much higher value for Cp. This means that Cp for any given solute in the herb will always be higher in the Tinofned extraction thereby resulting in a lower rate of dissolution compared to the tea extraction. Also as the dissolution proceeds the value of Cp increases which means the rate of dissolution is constantly reducing with time. This effect becomes more pronounced when the Cp concentration is higher as in the Tinofend extraction. Relative differences up to 5 times can been seen in some modelled examples using the concentration differences between the tea and Tinofend extractions.

One must also consider the effect the concentration will have on the viscosity of the extract solution, bearing in mind the plant nature of the herb and potential to extract water soluble polymeric carbohydrates, which will increase the viscosity. Taking starch, which is the most abundant storage polysaccharide in plants, as an example, a 1% solution of starch would have a viscosity in the region of 0.0035 PaS at 20 oC which compared to water at the same temperature is 0.00106. Such a change in viscosity would reduce the dissolution rate by over 3 times.

Overall although the Tinofend extraction time is 18 times longer than that of a typical tea, the fact that the temperature of Tinofned extraction is lower, the amount of water used is much lower and the surface area is lower will reduce the dissolution rate compared to a tea. We have seen potential reduction factors above of 1.15 times for the temperature effect, 14 times for the surface area effect, up to 5 times for the concentration effect and 3 times for a concentration induced viscosity effect.

These various effects would when combined reduce the rate of dissolution of the herb in the Tinofend extraction compared to a tea and therefore necessitate a longer treatment time compared to a Tea.

For the purpose of this comparison we are assuming a similar thickness of the concentration gradient (d) in both the Tinofend extraction and in making the tea, since stirring is the main factor to affect the value of d and no stirring is used in either case.

The chemical profile of Tinofend has been compared to that of a Tea (see report below) made using 2 g of the dried herb at a particle size between 0.8 to 2 mm in 240 ml of water at 100 oC for 10 minutes. The Tinofend sample was prepared using 2 g of Tinofend in 240 ml of water. As the Tinofend has an

extract ratio between 10:1 to 15:1 the concentration of the Tinofend solution would naturally be 10 to 15 times greater than the Tea solution. This is borne out by comparison of the 6 major peak heights in the HPLC chromatograms, where those of the Tinofend extraction, on average, were about 12 times greater than the same peaks in the tea Extraction. Overall the results show a similar chemical profile for the compounds extracted into the water in the tea to that of compounds in the rehydrated Tinofend, and a general equivalence of concentration when considering the extract ratio. If we reduce the 2 g of Tinofend used in the tests by the average increase in the concentration of 12 times, we get a value of 167 mg. So a single dose of about 167 mg of Tinofend would be equivalent to a single tea. As it would be reasonable to assume that a person could consume 3 teas a day it is therefore equally reasonable for 3 daily doses of 167 mg of Tinofend to be used.

Therefore on the basis that *Tinospora cordifolia* Teas are not Novel Foods and that Tinofend is, like a tea, a water extract and has a similar chemical profile, to that of a single tea, both qualitatively and quantitatively (at a single dose of 167 mg), we believe that Tinofend would not be classified as a Novel Food.

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Confidential

Project:

Comparison study of Tinospora Tea with Tinofend (TC aqueous extract powder) by HPLC and HPTLC fingerprinting.

Completed by:

PHPL ADIC dept - Ganesh Saste, Chetana Ghule, Dr Aboli Girme (Senior Manager)

Procedure:

Tinospora Tea – Dried stems of *Tinospora cordifolia* (TC) were grinded in powder (mesh size 10# [2.00 mm] -20 # [0.841 mm]). The 2 grams this powdered raw material was taken in 240ml water and boiled at 100 °C for 10 minutes. The resulting solution was centrifuged and filtered. The filtrate was used further for HPLC and HPTLC analysis as per the validated in-house testing method.

Tinofend (TC aqueous extract powder): It was prepared as per the sample preparation method defined in the in-house method and using 2 g Tinofend powder per 240 ml.

HPLC-Instrument- Shimadzu UFLC and Nexera X2 systems

Mobile phase- Solvent A (0.1% phosphoric acid in water) and solvent B (acetonitrile) with gradient phase

Column-Phenomenex C18 column

HPTLC-Instruemnt- TLC applicator Linomat 5 (Camag, Switzerland) equipped with vision CATS software (version 3.0.20196) and TLC visualizer 2 (Camag, Switzerland).

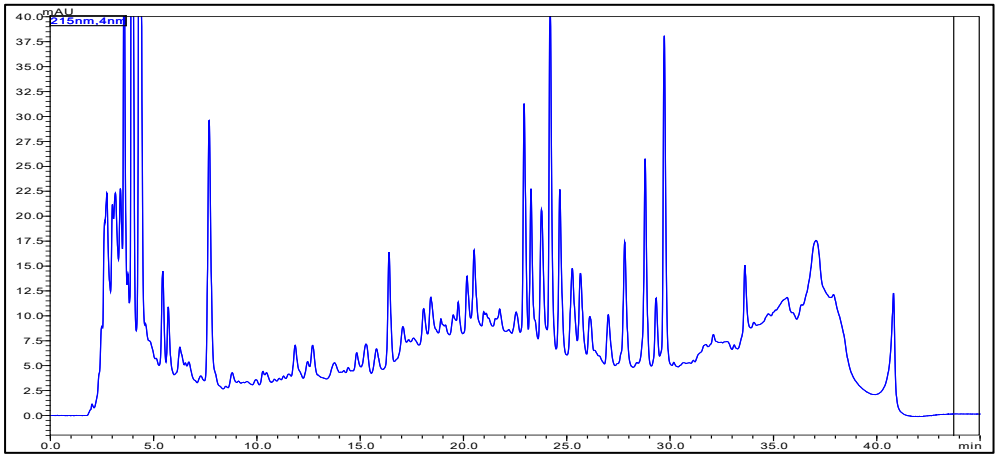
Report Date: April 2021

Results:

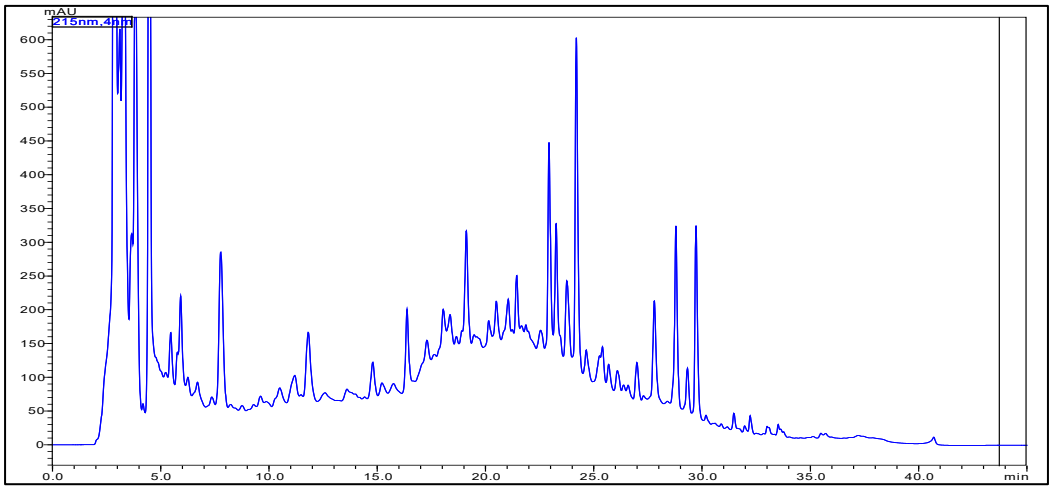
Sample Name	Sample	Sample solution after process
Tinospora Tea		
Tinofend (TC aqeous extract)		

HPLC Fingerprinting analysis:

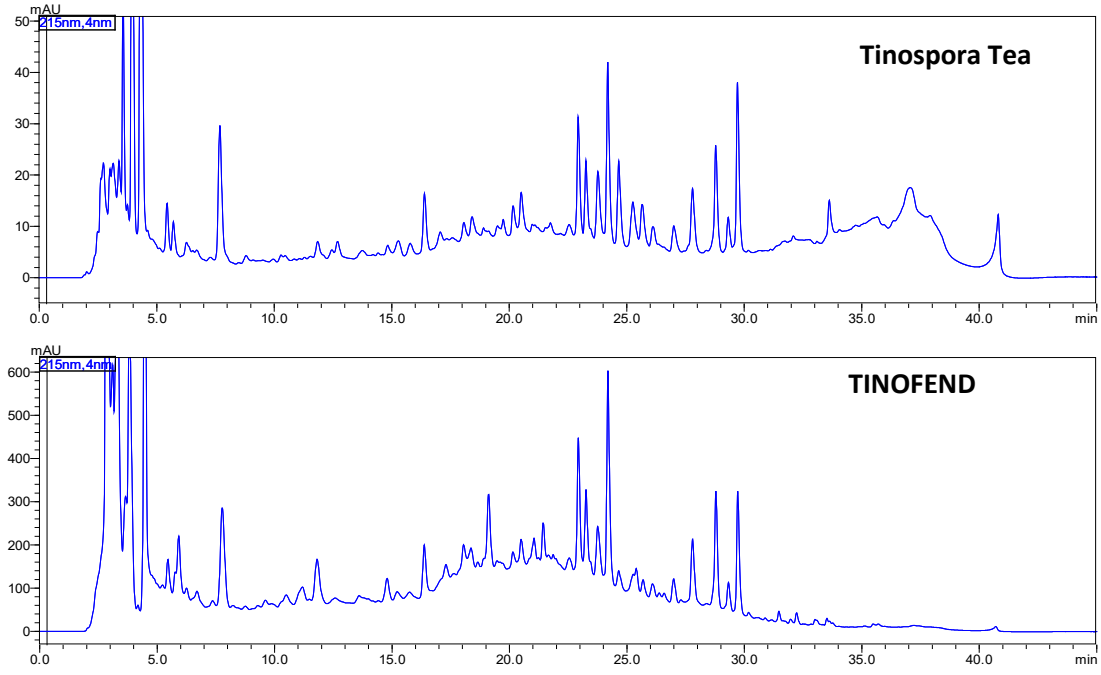
1) Tinospora Tea:



2) Tinofend:



3) Stack chromatograms (Tinospora Tea & Tinofend):



HTPLC Fingerprinting analysis:

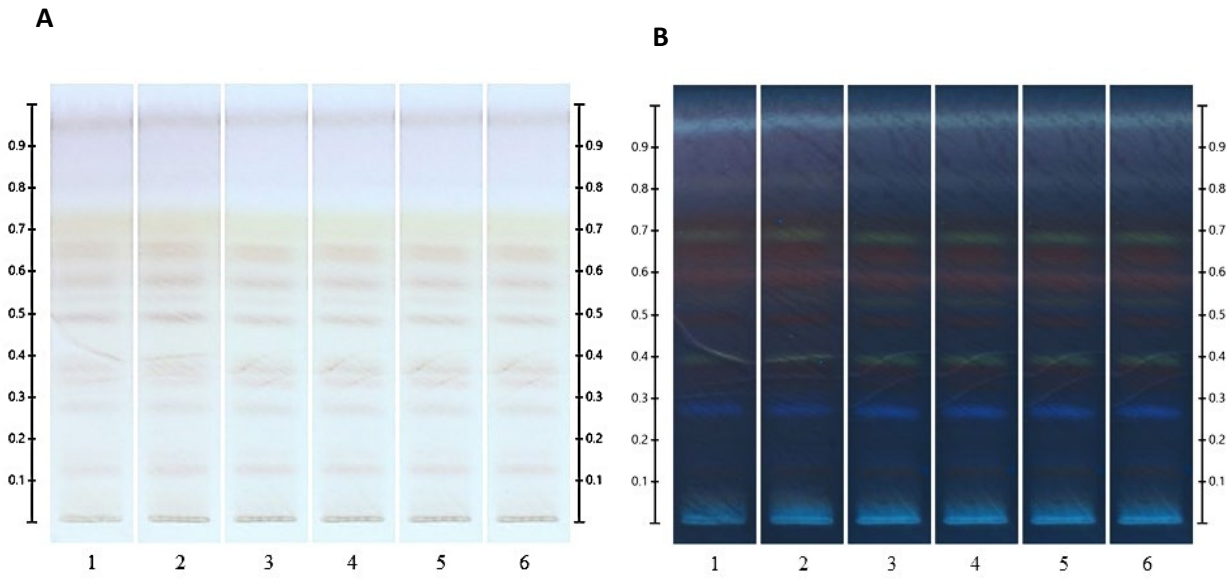


Figure: HPTLC profiles of Tinospora Tea and Tinofend after derivatization with anisaldehyde sulphuric acid reagent (ASR) under white light (A), UV 366nm (B)

Tracks 1-2 = Tinospora Tea (3 & 4 µl)

Tracks 3-4 = Tinofend extract (3 µl)

Tracks 5-6 = Tinofend (4 µl)

Conclusion:

Using validated in house HPLC method the HPLC fingerprinting showed the similar peak profile for both the samples i.e. Tinospora Tea and Tinofend between the retention time (RT) 6-7 minutes, 22-24, and 31-32 minutes at 215 nm.

Using In-House HPTLC method developed for fingerprinting, the HPTLC profile of the Tinospora Tea and Tinofend found prominent reddish Rf zones at 0.28, 0.50, 0.58, and light red at 0.65 Rf after derivatization with ASR in visible light. And at 366 nm a major blue quenching zone bands were found in both the sample at 0.28 Rf and Greenish bands at 0.39, 0.70 Rf and Reddish zone at O.58 and 0.65 Rf.

This report confirms that HPLC and HPTLC chromatographic fingerprints of Tinospora Tea when boiled in water at 100 ℃ are similar to that of Tinofend (aqueous TC extract).