Authentication of neutral henna leaves by HPTLC

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Introduction

Neutral henna (*Cassia italica*) is a traditional plant used in cosmetics for hair care applications or in medicine as laxative. We chose to develop a plant extract for cosmetic application from neutral henna leaves.
Identification of neutral henna leaves is difficult because there is no monograph. Thanks to HPTLC, a method has been developed for the identification of the vegetal material through a phytochemical profile. The analysis of polyphenols [1] has been realised before and after hydrolysis of the extract.

Thereby, the raw material can be validated according to an internal reference profile, established with the plant used to develop the extract in the first step. The method has been tested on plants from different suppliers. According to them, leaves could have different aspects (whole or ground into fine powder). After extraction of these raw materials, the extracts were applied for validation in HPTLC.

Materials and Methods

Samples preparation :

- Extract preparation : Extraction of leaves by a mixture of propylene glycol + water followed by a filtration
- Hydrolysed sample preparation : 5mL of extract + 5 mL HCl during 1h at 100°C. Then the mix is shaken with 10mL Ethyl Ac etate. The upper phase is evaporated to dryness and the residue is dissolved with 0,5mL methanol
- Non hydrolysed sample preparation : 5mL of extract is evaporated to dryness, the residue is dissolved with 0,5 mL methanol
- Standards : 1 mg in 1 mL methanol

HPTLC conditions :

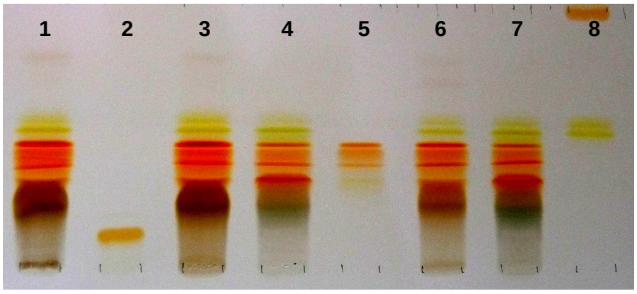
- HPTLC plates silica gel 60 F254s (Merck), 20 x 10 cm
- Mobile phase : System 1 – Toluene / Ethyl Acetate / Acetic Acid 50:40:10 (v/v/v)
  System 2 – Ethyl Acetate / Methyl Ethyl Ketone / Formic Acid / Water 25:15:5:5 (v/v/v/v)
- Migration distance : 70 mm for system 1 and then 35 mm for system 2
- Post chromatographic derivatization : dipping for 1 s into the NEU reagent then into the PEG reagent
- Observation : UV at 366 nm and Visible

Results and Discussion

Application of hydrolysed samples

Migration : System 1 then system 2
Observation : Visible

1 : supplier 1 whole leaves – batch 1
2 : rutin
3 : supplier 1 whole leaves – batch 2
4 : supplier 2 leaf powder
5 : quercetin
6 : Internal reference whole leaves
7 : supplier 3 leaf powder
8 : kaempferol



Presence of quercetin and kaempferol in all extracts.

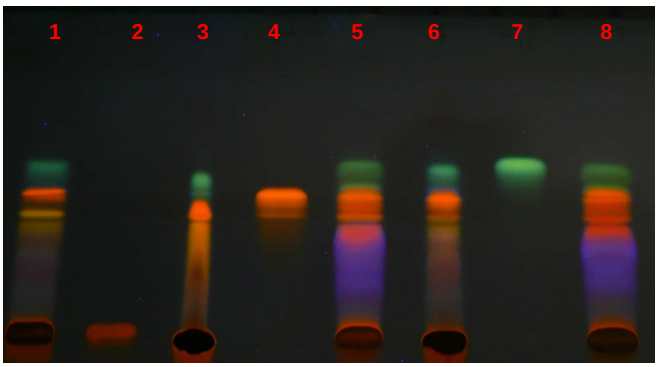
At Rf=0,8, a weak orange spot is present in the extracts n°1, 3 and 6. It is absent in extracts n°4 and 7.

At Rf=0,2, a blue-green spot appeared in extracts n°4 and 7. It is absent in extracts n°1, 3 and 6.

Application of non-hydrolysed samples

Migration : System 1
Observation : UV under 366 nm

1 : supplier 1 whole leaves – batch 1
2 : rutin
3 : supplier 1 whole leaves – batch 2
4 : quercetin
5 : supplier 2 leaf powder
6 : Internal reference whole leaves
7 : kaempferol
8 : supplier 3 leaf powder



Presence of quercetin and kaempferol in all extracts.

At Rf=0,3, a blue fluorescent spot is visualized in extracts n°5 and 8. It is missing in the extract n°1, 3 and 6.

The extracts made with whole leaves (n°1, 3 and 6) are similar to each other. They have the same profile that the internal reference (n°6). The extracts realized from powder have a different pattern with additional or missing spots compared to the internal profile.
These kinds of observations suggest that the powders are not composed by only neutral henna leaves. The powders are probably mixed with another plant or with other neutral henna parts (like seed, flower, stem…).
Thanks to HPTLC analysis, the supplier 1 has been chosen.

Conclusion

HPTLC is thus a good and quick method to highlight the falsification of plant material. In our case, HPTLC allows to choose the right supplier according to our required specifications

Reference

[1] : El-Sayed N.H. *et al* ; 1992 ; Phytochemistry ; Vol 31 ; p2187