

# Comparative study of high-performance thin-layer chromatography and antioxidant potential of *Hydrocotyle asiatica* mother tincture used in homoeopathy

Rakhi Mishra<sup>1,2\*</sup>, Binit Kumar Dwivedi<sup>2</sup>, Deepshikha Gupta<sup>1</sup>

<sup>1</sup>Department of Chemistry, Amity Institute of Applied Sciences, Amity University, Uttar Pradesh, India, <sup>2</sup>Drug Standardization Unit, Dr. D.P. Rastogi Central Research Institute for Homoeopathy, Noida, Uttar Pradesh, India

## Abstract

**Background:** *Hydrocotyle asiatica* has a therapeutic significance in the Indian system of medicine due to its rich antioxidant activity. In Homoeopathy, *Hydrocotyle asiatica* is used for the treatment of jaundice, skin diseases, dropsy, elephantiasis, leprosy, gonorrhoea, leucorrhoea, and nervous debility. It contains the abundant triterpene glycoside Asiatic acid which shows cytotoxic activity on cancer cells. Its homoeopathic mother tincture is a major source of antioxidant compounds, which is responsible for its overall pharmacological activity. **Objectives:** This study was done to evaluate antioxidant activity and High-Performance Thin-Layer Chromatography study of *Hydrocotyle asiatica* in-house homoeopathic mother tincture and market samples. **Materials and Methods:** Antioxidant activity of in-house homoeopathic mother tincture (A) and three market samples (B, C, and D) were determined by 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free-radical scavenging activity, total phenol, and 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay methods. High-performance thin-layer chromatography (HPTLC) study performed on precoated silica gel 60 F<sub>254</sub> TLC plate, mobile phase used was toluene: ethyl acetate: formic acid (5.5:4.5:1, v/v/v) and UV detection were performed at 254 and 366 nm. For derivatization, anisaldehyde sulfuric acid reagent was used. **Results:** The homoeopathic mother tincture of *Hydrocotyle asiatica* had prominent antioxidant activity. HPTLC study indicated the presence of triterpene glycoside compound Asiatic acid in chloroform extract of *Hydrocotyle asiatica*. **Conclusion:** The mother tinctures prepared by authenticated plant samples showed maximum active constituents and prominent antioxidant activity as compared to the mother tinctures procured from the market. The present study justifies the homoeopathic usage of *Hydrocotyle asiatica* and highlights its healing properties.

**Keywords:** 2,2'-Azino-bis 3-ethylbenzothiazoline-6-sulfonic, 2,2-Diphenyl-1-picryl-hydrazyl-hydrate, Antioxidant activity, Homoeopathic mother tincture, High-performance thin-layer chromatography, *Hydrocotyle asiatica*

## INTRODUCTION

*Hydrocotyle asiatica* (*Centella asiatica*) belongs to the family of perennial plants *Umbelliferae* (*Apiaceae*).<sup>[1]</sup> It is a tasteless, odorless plant that thrives in and around water [Figure 1].<sup>[2]</sup> *Hydrocotyle asiatica* is a very important medicinal herb used in different orient and is becoming a popular medicine in the west.<sup>[3,4]</sup> *Hydrocotyle asiatica* is commonly known as *brahmi* in Hindi, *mundukparni*, *kodavanin* Ayurveda, *Gotu kola* in China and Sri Lanka, *buakbok* in Thailand, *kaki kuda* in Indonesia, and *yuhong-yuhong* in the Philippines.<sup>[5-7]</sup> In Chinese medicine, *Hydrocotyle asiatica* is known as one of the 'miracle elixirs of life' known over 2000 years ago.<sup>[8]</sup> It is native to wetlands in Asia such as India, Sri Lanka, China, Indonesia, Malaysia, South Africa, and Madagascar.<sup>[9]</sup> Its active constituents

include pentacyclic triterpene derivatives.<sup>[10]</sup> *Hydrocotyle asiatica* is an important medicinal plant that is widely used as a homoeopathic medicine due to its bioactive compounds such as Asiatic acid, rutin, kaempferol, quercetin, gallic acid, luteolin, and catechin.<sup>[11,12]</sup> It has been used in folk herbal medicine for centuries for memory enhancement, depression, wound healing, psoriasis, and the treatment of related chronic

**\*Address for correspondence:** Rakhi Mishra, Dr. D.P. Rastogi Central Research Institute for Homoeopathy, A-1/1, Sector-24, Noida, Uttar Pradesh - 201301, India. E-mail: rakhimishra020@gmail.com

**Received:** 11 November 2021; **Accepted:** 18 August 2022

Access this article online	
<b>Quick Response Code:</b>	<b>Website:</b> www.ijrh.org
Available in print version only	<b>DOI:</b> 10.53945/2320-7094.1102

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**How to cite this article:** Mishra R, Dwivedi BK, Gupta D. Comparative study of high-performance thin-layer chromatography and antioxidant potential of *Hydrocotyle asiatica* mother tincture used in homoeopathy. Indian J Res Homoeopathy 2022;16(3):200-210.



**Figure 1:** *Hydrocotyle asiatica* (a) plant, (b) part used (dried whole plant)

diseases.<sup>[13,14]</sup> *Hydrocotyle asiatica* is believed to possess diverse pharmacological actions such as neuroprotective, nerve regenerative, immunomodulatory, antidepressive, memory-enhancing, gastroprotective, cardioprotective, radioprotective, anti-cancer, antimicrobial, anti-inflammatory, anti-diabetic, and antioxidative.<sup>[15]</sup> *Hydrocotyle asiatica* is especially recognized for its ability to revitalize nerves and brain cells.<sup>[16]</sup>

As per the book on *Materia Medica* by William Boericke, *Hydrocotyle asiatica* is used as a curative in the disorders that exhibit interstitial inflammation, cellular proliferation, leprosy, lupus, granular ulceration of the womb, profuse leucorrhoea, psoriasis gyrate, and syphilitic infections.<sup>[17]</sup> *Hydrocotyle asiatica* contains the most abundant triterpene glycoside Asiatic acid which shows cytotoxic activity on cancer cells. Asiatic acid derivative synthesis can be used as an anti-cancer agent.<sup>[18]</sup> It also has a strong neuroprotective effect due to the presence of Asiatic acid bioactive compound.<sup>[19-21]</sup> Asiatic acid is a triterpene glycoside and is commonly used for wound healing. Asiatic acid has antioxidant, anti-inflammatory, and neuroprotective properties.<sup>[22]</sup> Chromatography is a useful analytical method used for the qualitative authentication and evaluation of plant extracts.

In the present study, high-performance thin-layer chromatography (HPTLC) was carried out to identify and confirm the presence of the bioactive compound, Asiatic acid in *Hydrocotyle asiatica* homoeopathic mother tincture. Therefore, this study was done to investigate the presence of Asiatic acid in the in-house mother tincture sample (A) and three market samples (B, C, and D) of *Hydrocotyle asiatica* by HPTLC method. Furthermore, the whole plant of *Hydrocotyle asiatica* is a rich source of antioxidant compounds.<sup>[23]</sup> Antioxidant components are micro constituents that inhibit lipid oxidation by inhibiting the initiation or propagation of oxidizing chain reactions and are involved in the scavenging of free radicals.<sup>[24]</sup> In view of that, we designed the study to evaluate the antioxidant potential of *Hydrocotyle asiatica* by various assay methods. The present study is helpful for the determination and quantification of antioxidant compounds which are useful for producing *Hydrocotyle asiatica*-based drugs for the treatment of various ailments of human beings.

## METHODS

### Collection of plant materials

The whole plant of the specimen *Hydrocotyle asiatica* was collected and authenticated by staff at the Center of

Medicinal Plants Research in Homoeopathy, Emerald, Tamil Nadu, India. The voucher specimen was deposited in the herbarium and in the laboratory of Dr. D. P. Rastogi Central Research Institute (Homoeopathy) Noida, Uttar Pradesh, India, for future reference with collection number 9647. The whole plant was shade-dried and powdered mechanically, and the fine powder was used for the preparation of the mother tincture. Asiatic acid ( $C_{30}H_{48}O_5$ , M. P. 325–330°C) with purity by high performance liquid chromatography (HPLC) >98% w/w purchased from Sigma Aldrich, USA. Solvents used were ethanol, methanol, HPLC water, and chloroform of analytical grade purity (Merck Ltd., India).

### Physicochemical studies for raw drug standardization

#### Loss on drying

Loss on drying method was used for determination of moisture content as per methods recommended in Homoeopathic Pharmacopeia of India.<sup>[25]</sup> Percentage loss on drying was calculated.

#### Foreign matter determination

For foreign matter determination, 100 g of plant raw material was taken and outspread in the form of a thin layer. The sample was examined by a 6× lens or with an unaided eye, the foreign organic matter was picked manually. The ratio of total foreign matter weighed, and the weight of drug taken gave the % of foreign matter.

#### Total ash value determination

In the drug, the impurity present in the form of organic matter was determined with the help of the total ash value. For its determination, 2 g of the dried raw drug was weighed in powdered form in a pre-weighed silica crucible. The sample was incinerated in a silica crucible by gradually increasing the temperature up to 450°C for 4 h or until it became carbon-free. The crucible was cooled and weighed until a constant weight was obtained. Percent of total ash value was then calculated by taking the ratio of loss in weight to the weight of the sample taken.

#### Acid-insoluble ash value determination

After total ash value determination, 25 mL of 5 M hydrochloric acid was added to the dried ash and boiled in a water bath for 10 min. The solution was concentrated till its color changed to yellow. Acid insoluble matter was filtered using ashless Whatman paper number 1 followed by washing with distilled water. The paper was again ignited in a crucible at a temperature not more than 450°C for 4 h, after which the crucible was kept in a desiccator, cooled, and weighed. With reference to the originally taken air-dried powdered drug, the percentage of acid-insoluble ash value was calculated.<sup>[26]</sup>

#### Water-soluble extractive value determination

For determination of water extractive value, 2 g of sample was accurately weighed, and air-dried powdered drug was put in a conical flask with 100 mL water added to it. The

solution was allowed to stand for 24 h with intermittent shaking of the flask after every 4 h. The water-soluble extractive was filtered using Whatman filter paper. 25 mL of this filtrate was completely dried on a pre-weighed Petri plate at 105°C. The increase in weight of the petri dish was noted to calculate the water-soluble extractive value determination. With reference to the originally taken air-dried powdered drug, the percentage of water-soluble extractive value was calculated.

#### Alcohol-soluble extractive value determination

For determination of alcohol soluble extractive value, accurately weighed 2 g air-dried powdered drug was put in a conical flask, and 100 mL absolute alcohol was added to it. The whole solution was left for 24 h for complete extraction at room temperature. The solution was shaken vigorously for a few minutes after every 6 h. The extract was filtered with the help of Whatman filter paper taking precautions to avoid evaporation loss of alcohol from the extract weighed the empty flat-bottomed Petri dish. The Petri dish with 25 mL of filtrate was heated at 105°C in an electric oven then cooled in a desiccator and weighed. With reference to originally taken air-dried powder, the percentage of alcohol-soluble extractive value was calculated and is shown in Table 1.

#### Preparation of crude extract/in-house mother tinctures

100 g of coarsely dried powdered *Hydrocotyle asiatica* whole plant was taken in which 300 mL distilled water and 730 mL strong alcohol (95%) were added to make one thousand milliliters of the mother tincture using the percolation method<sup>[27]</sup> (as per Homoeopathic Pharmacopoeia of India). This tincture was transferred to a tightly packed amber glass container and stored for further study.

**Table 1: Results of test of physicochemical properties of raw drug material**

Name of test	Result (%)
Foreign matter	2.00
Loss on drying	4.98
Total ash value	6.80
Acid-insoluble ash value	1.20
Water-soluble extractive value	26.82
Alcohol-soluble Extractive value	10.40

#### Qualitative phytochemical screening

Phytochemical tests were performed on crude extract for qualitative estimation of phytochemicals present in in-house mother tincture of *Hydrocotyle asiatica* with all respective testing procedures as described in the textbook by Harborne [Table 2].<sup>[28]</sup>

#### Standardization of mother tincture

Standardization of mother tincture was conducted to identify the organoleptic and physicochemical properties of mother tincture. Organoleptic properties measurement was done for color, odor, and clarity of solution. The samples were tested for various physicochemical properties such as sediments, pH, total solids, weight/mL, and total alcohol content.

#### Preparation of standard Asiatic acid

Dissolved 5 mg of Asiatic acid in 5 mL ethanol in a volumetric flask and sonicated for 10 min to prepare a working standard of asiatic acid with a concentration of 1 mg/mL.

#### Preparation of chloroform extract

25 mL of mother tincture was taken in a 50 mL beaker. The solution was evaporated on a water bath to remove the ethanol and extracted three times with 20 mL chloroform. Combined and concentrated chloroform was extracted up to 2 mL volume. TLC of chloroform extract of mother tincture was carried out and standard asiatic acid on silica gel 60 F<sub>254</sub> pre-coated plate was referenced.

#### HPTLC fingerprinting profile study

For HPTLC fingerprinting study a densitometric HPTLC Camag Linomat 5 (Switzerland) system was used.<sup>[31]</sup> As a sample applicator, Camag Linomat 5 was used for spotting TLC plate. Spots were made on silica gel 60 F<sub>254</sub> pre-coated plate (Merck) 20 × 10 cm plate with an aid of a sampling machine and the solvent front was run up to 70 mm height. For the development of the mobile phase, a saturating chamber Camag Twin Trough glass chamber was used. Camag TLC Scanner and software vision CATS were used for scanning purposes. HPLC grade solvents were used for all the extracts solutions. Volume applied for standard 2–6 µL and for sample 2–6 µL. Asiatic acid was used as a reference standard. For detection of triterpene glycoside Asiatic acid various mobile phase was used toluene: chloroform:ethanol (4:4:1, v/v/v), chloroform: methanol:formic acid (7:3:0.5, v/v/v) and chloroform: methanol (9:1, v/v), toluene: ethyl acetate: formic

**Table 2: Results of Phytochemical tests for screening of various phytochemicals present in mother tincture of *Hydrocotyle asiatica***

Name of phytochemical tests	Procedure	Observation	Result
Flavonoid (Lead acetate)	Mother tincture with a few drops of 10% lead acetate solution	Yellow precipitate	Positive
Alkaloids (Dragendroff's test)	In chloroform solution add dilute hydrochloric acid with a few drops of Dragendroff's reagent	Red-brown precipitate	Positive
Glycosides (Sodium hydroxide reagent test)	Mother tincture with 1 mL water and sodium hydroxide solution	Yellow color	Positive
Tannin (Ferric chloride test)	Mother tincture mixed with few drops of ferric chloride solution	Green color	Positive
Saponins (Foam test)	Mother tincture shaken with little quantity of water	Foam produced	Positive

acid (5:5:1, v/v/v). TLC spots were visualized after illumination at 254 nm, 366 nm, and after derivatization.

### Study of antioxidant potential

#### Determination of total phenolic content (TPC)

The TPC of the extracts was determined by Folin-Ciocalteu's reagent procedure reported by Singleton.<sup>[30]</sup> The total phenol content was estimated in *Hydrocotyle asiatica* in-house mother tincture sample A and its market samples (B, C and D). Ascorbic acid was used as the reference standard. Different concentrations (0.2661-8.517 mM) of ascorbic acid were prepared and analyzed at 736 nm and a calibration curve was plotted as absorbance versus concentration. TPC was estimated by using Ascorbic acid as standard approximately 50 µl of the mother tincture was mixed with 5 mL of 10% Folin-Ciocalteu's (phenol reagent) and 4 mL of sodium carbonate. The mixture was allowed to stand for 1 h in dark. After 1 h the color changed from yellow to blue. The absorbance of the solutions was measured at  $\lambda_{\max}$  736 nm using a UV-VIS spectrophotometer (U.V. Spectrophotometer SPECORD 200 plus Analytik Jena, Germany). The TPC was calculated from the calibration curve and in a result TPC of the *Hydrocotyle asiatica* sample A (in-house mother tincture) and market sample B, C, and D were calculated as the ascorbic acid equivalents (AAEs) using ascorbic acid as standard ( $Y=0.0753x+0.0256$ ,  $R^2=0.9995$ ). TPC was expressed in mM concentration of ascorbic acid equivalent.

#### 2,2-Diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging assay

The free radical scavenging activity of *Hydrocotyle asiatica* sample in-house mother tincture (A) and market sample (B, C, and D) was measured by DPPH radical scavenging assay. The standard solution of DPPH was prepared by dissolving 0.025 g in 25 mL methanol and different concentrations of standards/mother tincture sample (100 µL) were mixed with 4 mL methanol and 1 mL of DPPH standard. The mixture was allowed to stand for 1 h in dark, after which the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (UV Spectrophotometer SPECORD 200 plus Analytik Jena, Germany). The percentage inhibition was determined by comparing the result of the test and the control (methanol used as solvent blank).<sup>[31]</sup> Percentage degradation was calculated by the formula:

$$\text{DPPH radical scavenging (\%)} = \frac{[B - A] \times 100}{B}$$

Where,

A=Absorbance of the sample

B=Absorbance of control

The inhibiting effects of the mother tincture showed varied levels of DPPH radical scavenging activity, expressed as percentage degradation.

#### Determination of 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic (ABTS) assay

Free radical scavenging activity of in-house mother tincture sample (A) and market samples (B, C, and D) were determined

by ABTS radical cation decolorization assay. ABTS<sup>+</sup> cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1) stored in the dark at room temperature for 16 h before use. ABTS<sup>+</sup> solution was then diluted with methanol to obtain an absorbance of 0.700 at 746 nm. After the addition of 10 µL of mother tincture/standard in 2 mL of diluted ABTS<sup>+</sup> solution, the absorbance was measured at 5 min after the initial mixing. An appropriate solvent blank (methanol) was run in each assay.

Percent inhibition of absorbance at 746 nm was calculated using the formula:

$$\text{ABTS ion scavenging effect (\%)} = \frac{[(AB - AA) \times 100]}{AB}$$

Where,

AB is the absorbance of ABTS radical + methanol

AA is the absorbance of the ABTS radical+ sample/standard.

Trolox was used as a standard substance.

## RESULTS

### Results of physicochemical and phytochemical studies

The physicochemical properties of the tinctures of in-house drug sample (A) for parameters like sediments, pH, total solids, alcohol content, and weight per mL were analyzed and tabulated in Table 3. The results obtained for various physicochemical studies of raw drug are tabulated in Table 1. Phytochemical tests performed on the crude extract of the whole plant of *Hydrocotyle asiatica* showed positive results for various tests as mentioned in Table 2. Organoleptic observations of the prepared in-house mother tincture indicated the formation of a clear green solution with characteristic tincture odors.

### Result of HPTLC study

Based on extensive literature reviews, various combinations of solvent systems were studied with an aim to have an appropriate mobile phase composition for the best and most efficient HPTLC chromatographic separation of Asiatic acid in *Hydrocotyle asiatica* chloroform extract. In the mobile phase toluene: chloroform: ethanol (4:4:1, v/v/v), chloroform: methanol:formic acid (7:3:0.5, v/v/v), and chloroform: methanol (9:1, v/v) no appropriate resolution of the band was observed, whereas in mobile phase toluene: ethyl acetate: formic acid (5:5:1, v/v/v) efficient band resolution

**Table 3: Result of physicochemical properties of in-house sample (A) of *Hydrocotyle asiatica* mother tinctures**

Sl. No.	Parameter	In-house sample A
1.	Sediments	Nil
2.	pH	5.90
3.	Total solid	1.05% w/v
4.	wt/mL	0.90 g
5.	Alcohol content	64.0% v/v
6.	$\lambda_{\max}$	331 nm

**Table 4: Comparison of various mobile phase combinations used for preliminary screening study for best possible chromatographic separations of Asiatic acid**

Used mobile phase combinations for evaluation of asiatic acid	$R_f$ value	Observations
Toluene: chloroform: ethanol (4:4:1, v/v/v)	0.40	Poor resolution of band
Chloroform: methanol: formic acid (7:3:0.5, v/v/v)	0.10	No appropriate resolution of band
Chloroform: methanol (9:1, v/v/v)	0.23	No appropriate resolution of band
Toluene: ethyl acetate: formic acid (5:5:1, v/v/v)	0.49 for Asiatic acid	Efficient band resolution with improved $R_f$

of Asiatic acid was observed with improved  $R_f$  value of 0.49. Among all the mobile phase combinations studied, toluene: ethyl acetate: formic acid (5:5:1, v/v/v) was finalized to be the ideal one for the evaluation of compound Asiatic acid in *Hydrocotyle asiatica*. Thus, it was finalized the best appropriate mobile phase composition for the entire HPTLC method development study. Table 4 recorded various mobile phase combinations used for the preliminary screening study for the best possible separation of bands.

### Qualitative HPTLC study of in-house mother tincture and market samples

HPTLC study of *Hydrocotyle asiatica* chloroform extract of the in-house sample (A), three market samples (B, C, and D), and standard Asiatic acid was carried out using selected mobile phase toluene: ethyl acetate: formic acid in the ratio of volume (5:5:1, v/v/v). At UV light 254 nm and 366 nm, no spots of Asiatic acid were observed for any of the samples [Figures 2 and 3]. Therefore, for better resolution, an anisaldehyde-sulfuric acid reagent was used as derivatizing agent. After derivatizing the plate with anisaldehyde sulfuric acid reagent, a blue spot of Asiatic acid was observed at  $R_f$  0.49 [Figure 4] in in-house sample (A) and in the market sample (B, C, and D). 3D diagram of HPTLC dendrogram displayed the presence of standard Asiatic acid in mother tinctures of in-house sample A and market samples B, C, and D displayed in [Figure 5], respectively.

### Result of antioxidant activity

In the present study, the TPC of *Hydrocotyle asiatica* in-house sample A and its market sample B, C, and D were determined by Folin–Ciocalteu method and reported as AAE. The study reveals TPC found in *Hydrocotyle asiatica* in-house sample A, market samples B, C, and D (75 $\mu$ L) was 12.20, 2.80, 10.68, and 3.59 AAE [Table 5].

In the present study, the DPPH assay of *Hydrocotyle asiatica* in-house sample A and market samples B, C, and D were determined by DPPH radical scavenging assay method and reported as AAE. The study reveals *Hydrocotyle asiatica* in-house sample A and market samples B, C, and D were able to decolorize DPPH free radical, the DPPH scavenging increased with the concentration of the extract. The result showed a greater rate of DPPH scavenging activity found in the in-house sample as compared to the market samples. The percentage of inhibition found in 100  $\mu$ L volume of

**Table 5: Result of total phenolic content in mother tincture of *Hydrocotyle asiatica* in-house sample A and market samples B, C, and D**

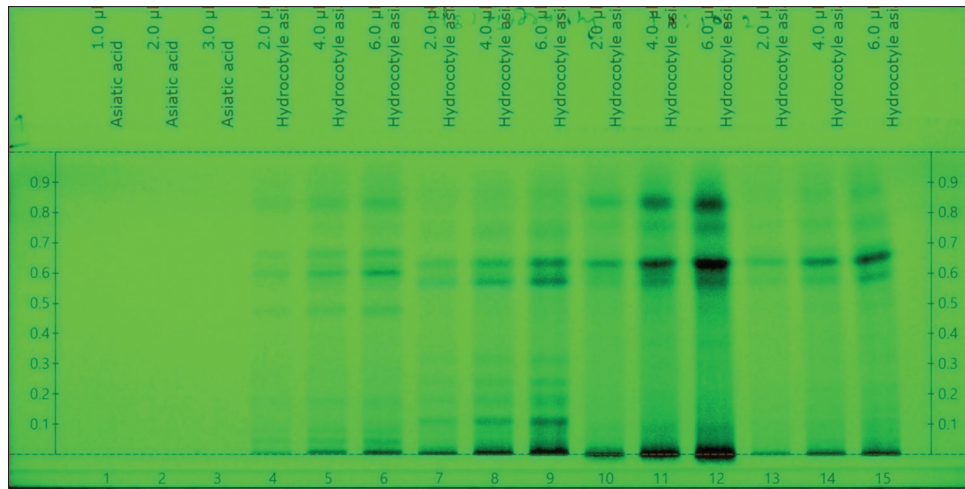
S. No.	Sample	Concentration in (mM) of AAE	Absorbance
1	<i>Hydrocotyle asiatica</i> in-house sample A	12.20	0.9439
2.	<i>Hydrocotyle asiatica</i> market sample B	2.80	0.2366
3.	<i>Hydrocotyle asiatica</i> market sample C	10.68	0.8298
4.	<i>Hydrocotyle asiatica</i> market sample D	3.59	0.2957
5.	Control	0.11	0.0172

AAE: Ascorbic acid equivalents

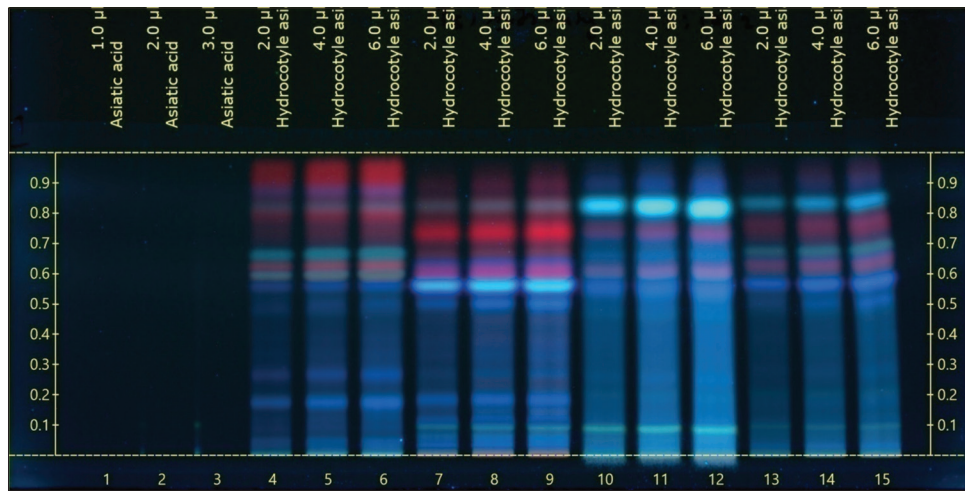
*Hydrocotyle asiatica* in-house sample A and market samples B, C, and D were 88.55%, 38.74%, 40.43%, and 24.10%, respectively [Table 6]. The order of DPPH scavenging against *Hydrocotyle asiatica* in-house sample A and market samples B, C, and D were found to be sample A > sample C > sample B > sample D.

In the DPPH assay, a significant correlation coefficient ( $R_f$  0.9955) was found between the antioxidant activity of alcoholic extracts (mother tinctures) of *Hydrocotyle asiatica* in-house sample A and market samples B, C, and D. The hydrogen radical scavenging action is known to be one of the important mechanisms for measuring antioxidant activity. This assay determines the scavenging of stable radical species DPPH by antioxidants compounds present in the mother tincture. The results showed a greater rate of DPPH scavenging activity in in-house sample A as compared to market samples B, C, and D probably due to the presence of high content of phenolic compound. Our study clearly indicated that the mother tincture of in-house sample A of *Hydrocotyle asiatica* exhibited high content of phenolic compound, that is, 12.20 mM which was significantly correlated with DPPH radical scavenging activity %, that is, 88.55% [Tables 5 and 6].

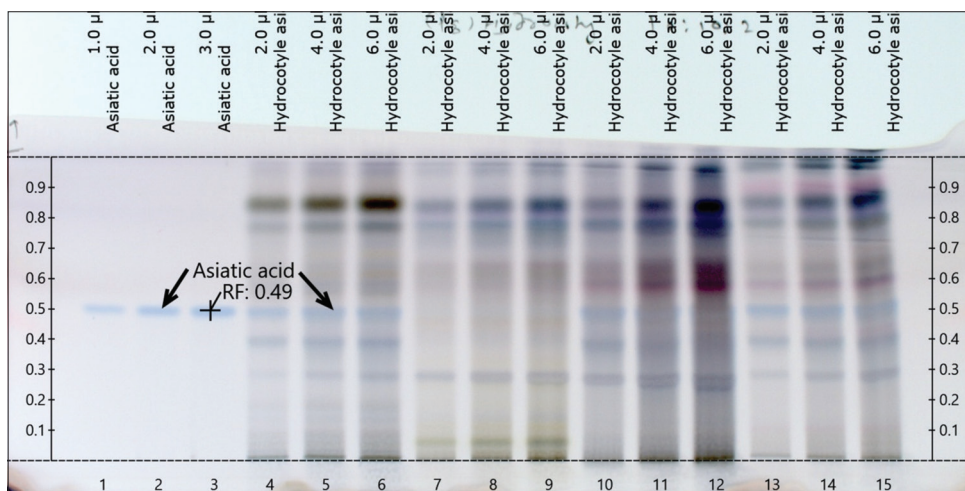
In the ABTS<sup>+</sup> assay of *Hydrocotyle asiatica*, in-house sample (A) and market sample (B, C, and D) were determined by ABTS<sup>+</sup> assay method and reported in terms of Trolox equivalents. A significant correlation coefficient (R, 0.9901) was found between the antioxidant activity of alcoholic extracts (mother tinctures) of *Hydrocotyle asiatica* in-house sample



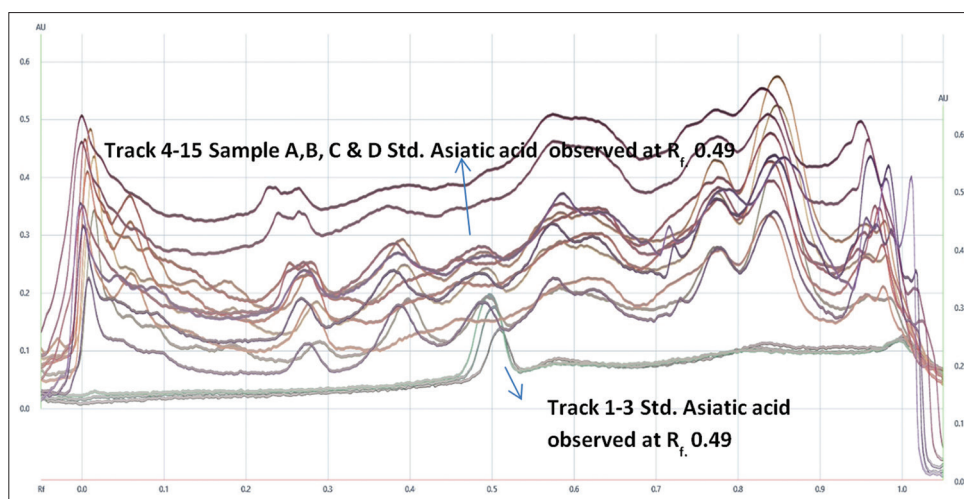
**Figure 2:** High-performance thin layer chromatography fingerprints of *Hydrocotyle asiatica* at ultraviolet 254 nm. Standard Asiatic acid Track (1-3), Track (4-6) In-house sample A color rendering index (h), Track (7-9) commercial market sample B, Track (10-12) market sample C, Track (13-15) market sample D



**Figure 3:** High-performance thin-layer chromatography fingerprints of *Hydrocotyle asiatica* at ultraviolet 366 nm. Standard Asiatic acid Track (1-3), Track (4-6) In-house sample A color rendering index (h), Track (7-9) commercial market sample B, Track (10-12) market sample C, Track (13-15) market sample D



**Figure 4:** High-performance thin layer chromatography fingerprints of *Hydrocotyle asiatica* after derivatization with anisaldehyde sulfuric acid reagent viewed in white light. Standard Asiatic acid Track (1-3), Track (4-6) In-house sample A color rendering index (h), Track (7-9) commercial market sample B, Track (10-12) market sample C, Track (13-15) market sample D



**Figure 5:** 3D view of high-performance thin-layer chromatography densitogram for chloroform extract of *Hydrocotyle asiatica* in-house sample A and market samples B, C, and D with respective standard (Track 1-3)

(A) and market sample (B, C, and D). The study reveals *Hydrocotyle asiatica* in-house sample (A) and market sample (B, C, and D) were able to decolorize ABTS<sup>+</sup> free radical, the ABTS radical cation scavenging activity increased with the concentration of the extract. The result showed the greater rate of ABTS cation scavenging activity found in *Hydrocotyle asiatica* in-house sample A as compared to the market sample (B, C, and D). The percentage of inhibition found in 10  $\mu$ L volume of *Hydrocotyle asiatica* in-house sample (A) and market sample (B, C, and D) were 99.89%, 99.40%, 99.82%, and 98.27%, respectively [Table 7]. The order of ABTS radical cation scavenging activity against *Hydrocotyle asiatica* was found to be in the order sample A > sample C > sample B > sample D.

## DISCUSSION

Several factors relating to climate, altitude, rainfall, and other conditions are responsible for the growth of the plant *Hydrocotyle asiatica* which affects the concentration of bioactive constituents grown in the same country. These conditions may produce significant variations in the bioactive constituents and thus cause a variation in the therapeutic efficacy. The variations in the different sample results may change the efficacy as the concentration of bioactive compounds is different in different samples analysed by us through HPTLC and UV spectrophotometer. The whole plant of *Hydrocotyle asiatica* is a natural source of important antioxidant substances. The results of various physicochemical studies of raw drugs tabulated in Table 1 indicate higher water-soluble extractive values as compared to alcohol-soluble extractive values. Since water is more polar than alcohol; therefore, all the polar compounds present in the plant are soluble in water. As a result, we found a greater extractive value in water as compared to alcohol. Whereas in the antioxidant study, high contents of phenolic compounds and a significant linear correlation between

**Table 6: Result of DPPH scavenging activity against *Hydrocotyle asiatica* in-house sample A and market samples B, C, and D**

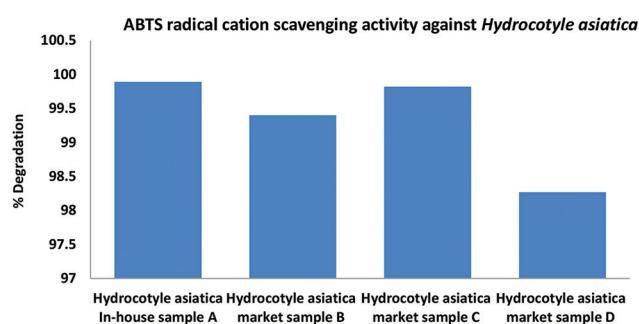
S. No.	Sample	Concentration in (mM)	Absorbance	% Degradation
1.	<i>Hydrocotyle asiatica</i> in-house sample A	9.14	0.2831	88.55%
2.	<i>Hydrocotyle asiatica</i> market sample B	4.35	1.5152	38.74%
3.	<i>Hydrocotyle asiatica</i> market sample C	8.98	0.3232	40.43%
4.	<i>Hydrocotyle asiatica</i> market sample D	2.95	1.8772	24.10%
5.	Control	0.63	2.4734	-

the values of the concentration of phenolic compounds and antioxidant activity indicate that these compounds contribute to the strong antioxidant activity of *Hydrocotyle asiatica*. Antioxidants are used to prevent aging, diabetes, heart diseases, cancer, and many other illnesses. The strong potential of tested *Hydrocotyle asiatica* mother tinctures as antioxidants in the present study suggests that the effect of *Hydrocotyle asiatica* mother tincture in the treatment of various diseases may be due to this whole plant antioxidant activity.

The present study revealed that as part of the preformulation study, the alcoholic extracts i.e., mother tincture of the whole plant of *Hydrocotyle asiatica* showed promising physicochemical characteristics. The result of the HPTLC fingerprinting profile study confirms the presence of triterpene glycoside Asiatic acid in chloroform extract of *Hydrocotyle asiatica* homoeopathic in-house mother tincture (A) as well as in commercial market samples (B, C, and D) at  $R_f = 0.49$ .

**Table 7: Result of ABTS radical cation scavenging activity against *Hydrocotyle asiatica* in-house sample A and market samples B, C, and D**

S. No.	Sample	Concentration in ( $\mu\text{g/mL}$ )	Absorbance	% Degradation
1.	<i>Hydrocotyle asiatica</i> in-house sample A	269.97	0.0003	99.89
2.	<i>Hydrocotyle asiatica</i> market sample B	266.42	0.0017	99.40
3.	<i>Hydrocotyle asiatica</i> market sample C	269.49	0.0005	99.82
4.	<i>Hydrocotyle asiatica</i> market sample D	258.75	0.0049	98.27
5.	Control	41.76	0.2832	-



The antioxidant activity of *Hydrocotyle asiatica* in-house mother tincture and the commercial market sample was investigated by different antioxidant assay methods such as total phenol, DPPH, and ABTS assay. Results show that the antioxidant potential of *Hydrocotyle asiatica* mother tincture demonstrated the highest antioxidant activity found in in-house mother tincture sample as compared to the market samples. The present study demonstrates that the studied homeopathic mother tincture of *Hydrocotyle asiatica* has a significant concentration of polyphenols. The high polyphenol content correlated with the significant antioxidant activity and can be the explanation for the beneficial effect of *Hydrocotyle asiatica* mother tincture in homeopathic treatments.

## CONCLUSION

The present study may push forth further research work to increase the usefulness of the plant *Hydrocotyle asiatica* in alternative systems of medicine. Quantitative estimation of other compounds present in this plant may also be evaluated in future studies responsible for its other pharmacological activities. Further, it is highlighted that the mother tinctures prepared by authenticated plant samples bear better quantity and quality of active constituents and thus displayed more prominent antioxidant activity, in this case.

## ACKNOWLEDGMENT

The authors would like to express their thanks of gratitude to Dr. Anil Khurana, former Director General, Central Council for Research in Homoeopathy, New Delhi, and Dr. Jaya Gupta, former In-charge, Dr. D. P. Rastogi Central Research Institute of Homoeopathy Noida for their support and cooperation.

## Financial support and sponsorship

Nil.

## Conflicts of interest

None declared.

## REFERENCES

- Menglan S, Fading P, Zehui P, Watson MF, Cannon FM, Holmes-Smith I, et al. *Apiaceae, Umbelliferae*. Flora China 2005;14:1-205.
- Gohil KJ, Patel JA, Gajjar AK. Pharmacological Review on *Centella asiatica*: A potential herbal cure-all. Indian J Pharm Sci 2010;72: 546-56.
- Bown D. Encyclopedia of Herbs and their Uses the Herb Society of America. London: Dorling Kindersley; 1995. p. 361-5.
- Chevallier A. The Encyclopedia of Medicinal Plants. London: Dorling Kindersley; 1996. p. 257.
- Ministry of Health and Family Welfare Government of India. Homoeopathic Pharmacopoeia of India. *Hydrocotyle asiatica*. Vol. 1. New Delhi: Ministry of Health; 1971. p. 218.
- Yao CH, Yeh JY, Chen YS, Li MH, Huang CH. Wound-healing effect of electrospun gelatin nanofibres containing *Centella asiatica* extract in a rat model. J Tissue Eng Regen Med 2017;11:905-15.
- Ravi A, Khan S, Suklabaidya M, Priyadurairaj P, Sudarsanan PS, Chandrasekar K. Antioxidant activity and *in silico* analysis of *Centella asiatica* and *Indigofera aspalathoides* in psoriasis. Biomed Pharmacol J 2018;11:1403-12.
- Diwan PC, Karwande I, Singh AK. Anti-anxiety profile of mandukaparni *Centella asiatica* Linn in animals. Fitoterapia 1991;62:255-7.
- Sabaragamuwa R, Perera CO, Fedrizzi B. *Centella asiatica* (Gotu kola) as a neuroprotectant and its potential role in healthy aging. Trends Food Sci 2018;79:88-97.
- Brinkhaus B, Lindner M, Schuppan D, Hahn EG. Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*. Phytomedicine 2000;7:427-48.
- Joseph GV, Chaturvedi S, Deokule SS. Standardisation and quality evaluation of *Centella asiatica* Linn. Anc Sci Life 2001;20:99-110.
- Borhan MZ, Ahmad R, Rusop M, Abdullah S. Green extraction: Enhanced extraction yield of Asiatic acid from *Centella asiatica* (L.) nanopowders. J Appl Chem 2013;2013:460168.
- Gray NE, Magana AA, Lak P, Wright KM, Quinn J, Stevens JF, et al. *Centella asiatica*: Phytochemistry and mechanisms of neuroprotection and cognitive enhancement. Phytochem Rev 2018;17:161-94.
- Puttarak P, Dilokthornsakul P, Saokaew S, Dhippayom T, Kongkaew C, Sruamsiri R, et al. Effects of *Centella asiatica* (L.) Urb. on cognitive function and mood-related outcomes: A systematic review and meta-analysis. Sci Rep 2017;7:10646.
- Orhan IE. *Centella asiatica* (L.) Urban: From traditional medicine to modern medicine with neuroprotective potential. Evid Based Complement Altern Med 2012;946259.
- Seevaratnam V, Banumathi P, Premalatha MR, Sundaram SP, Arumugam T. Functional properties of *Centella asiatica* (L.): A review. Int J Pharm Pharm Sci 2012;4:8-14.
- Boericke W. Homoeopathic Materia Medica. 9<sup>th</sup> ed. *Hydrocotyle asiatica*; 2000. Available from: <https://www.homeoint.org/books/boericmm> [Last accessed on 2021 Sep 20].
- Jana U, Sur TK, Maity LN, Debnath PK, Bhattacharyya D. A clinical study on the management of generalized anxiety disorder with *Centella asiatica*. Nepal Med Coll J 2010;12:8-11.
- Kumar A, Prakash A, Dogra S. *Centella asiatica* attenuates D-galactose-



- induced cognitive impairment, oxidative and mitochondrial dysfunction in mice. *Int J Alzheimers Dis* 2011;2011:347569.
20. Tabassum R, Vaibhav K, Shrivastava P, Khan A, Ahmed ME, Javed H, *et al.* *Centella asiatica* attenuates the neurobehavioral, neurochemical and histological changes in transient focal middle cerebral artery occlusion rats. *Neurol Sci* 2013;34:925-33.
  21. Xu CL, Qu R, Zhang J, Li LF, Ma SP. Neuroprotective effects of madecassoside in early stage of Parkinson's disease induced by MPTP in rats. *Fitoterapia* 2103;90:112-8.
  22. Asiatic Acid, Anti-Inflammatory Agent. Available from: <https://www.adooq.com/asiatic-acid.html> [Last accessed on 2021 Sep 25].
  23. Azmin SN, Shukri MN. Chemical fingerprint of *Centella asiatica*'s bioactive compounds in the ethanolic and aqueous extracts. *Adv Biomark Sci Technol* 2020;2:35-44.
  24. Irshad M, Zafaryab M, Singh M, Rizvi MM. Comparative analysis of the antioxidant activity of *Cassia fistula* extracts. *Int J Med Chem* 2012;2012:157125.
  25. Ministry of Health and Family Welfare Government of India. Homoeopathic Pharmacopoeia of India. Vol. 1. New Delhi: The Controller of Publications Government of India, Ministry of Health and Family Welfare; 1971. p. 243-5.
  26. Mishra R, Kumar M, Dwivedi B, Arya B, Arya R, Khurana A, *et al.* Chemoprofiling of Homoeopathic drug *Coffea cruda*. *Eur J Biomed Pharm* 2018;5:276-81.
  27. Ministry of Health and Family Welfare Government of India. Homoeopathic Pharmacopoeia of India. Vol. 10. New Delhi: The Controller of Publications, Ministry of Health and Family Welfare, Government of India; 2013. p. 96-7.
  28. Harborne J. The terpenoids. In: *Phytochemical Methods*. Berlin: Springer; 1973. p. 89-131.
  29. Reich E, Schibli A. High-Performance Thin Layer Chromatography for the Analysis of Medicinal Plants. New York: Thieme Medical Publishers Inc.; 2007. p. 67.
  30. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-ciocalteu reagent. *Methods Enzymol* 1999;299:152-78.
  31. Gupta D. Methods for determination of antioxidant capacity: A review. *Int J Pharm Sci Res* 2015;6:546-66.

## Études comparatives de la chromatographie en couche mince haute performance et du potentiel antioxydant de la teinture mère d'*Hydrocotyle asiatica* utilisée en homéopathie

**Contexte:** *Hydrocotyle asiatica* a une grande importance thérapeutique dans le système de médecine indien en raison de sa riche activité antioxydante. En homéopathie, l'*Hydrocotyle asiatica* est utilisée pour le traitement de la jaunisse, des maladies de la peau, de l'hydropisie, de l'éléphantiasis, de la lèpre, de la gonorrhée, de la leucorrhée et de la débilité nerveuse. Il contient le glycoside triterpénique le plus abondant, l'acide asiatique, qui présente une activité cytotoxique sur les cellules cancéreuses. La teinture mère homéopathique de ce médicament est une source importante de composés antioxydants, qui sont responsables de son activité pharmacologique globale. **Objectifs:** L'objectif de l'étude était d'évaluer l'activité antioxydante et l'étude par chromatographie en couche mince à haute performance (HPTLC) de la teinture mère homéopathique interne d'*Hydrocotyle asiatica* et des échantillons du marché. **Matériaux et méthodes:** L'activité antioxydante de la teinture mère homéopathique maison (A) et de trois échantillons du marché (B, C et D) a été déterminée par les méthodes suivantes : activité de piégeage des radicaux libres du 2,2-diphényl-1-picryl-hydrazyl-hydrate, phénol total et dosage du 2,2-azino-bis-(3-éthylbenzothiazoline-6-sulfonate). L'étude HPTLC a été réalisée sur une plaque TLC pré-enduite de gel de silice 60 F254, la phase mobile utilisée était toluène:acétate d'éthyle:acide formique (5,5:4,5:1, v/v/v) et la détection UV a été réalisée à 254 et 366 nm. Pour la dérivation, un réactif à base d'anisaldéhyde et d'acide sulfurique a été utilisé. **Résultats:** Le résultat a révélé que le test antioxydant de la teinture mère homéopathique d'*Hydrocotyle asiatica* avait une activité antioxydante importante. L'étude HPTLC a indiqué la présence d'un composé glycoside triterpène, l'acide asiatique, dans l'extrait chloroforme d'*Hydrocotyle asiatica*. **Conclusion:** Les teintures mères préparées à partir d'échantillons de plantes authentifiées ont montré un maximum de composants actifs et une activité antioxydante importante par rapport aux teintures mères achetées sur le marché. La présente étude justifie l'usage homéopathique d'*Hydrocotyle asiatica* et met en évidence ses propriétés curatives.

## Vergleichende Studien zur Hochleistungs-Dünnschichtchromatographie und zum antioxidativen Potenzial der in der Homöopathie verwendeten Urtinktur von *Hydrocotyle asiatica*

**Hintergrund:** *Hydrocotyle asiatica* hat im indischen Medizinsystem aufgrund seiner reichhaltigen antioxidativen Aktivität große therapeutische Bedeutung. In der Homöopathie, *Hydrocotyle asiatica* wird zur Behandlung von Gelbsucht, Hautkrankheiten, Wassersucht, Elephantiasis, Lepra, Gonorrhöe, Leukorrhöe und Nervenschwäche verwendet. Es enthält das am häufigsten vorkommende Triterpenglykosid Asiasäure, das eine zytotoxische Wirkung auf Krebszellen hat. Die homöopathische Urtinktur dieses Arzneimittels ist eine wichtige Quelle für antioxidative Verbindungen, die für seine allgemeine pharmakologische Aktivität verantwortlich sind. **Zielsetzungen:** Ziel der Studie war es, die antioxidative Aktivität und die Hochleistungs-Dünnschichtchromatographie (HPTLC)-Studie von *Hydrocotyle asiatica* als hauseigene homöopathische Urtinktur und Marktproben zu bewerten. **Materialien und Methoden:** Die antioxidative Aktivität der hauseigenen homöopathischen Urtinktur (A) und dreier Marktproben (B, C und D) wurde mittels 2,2-Diphenyl-1-picryl-hydrazyl-hydratfreier Radikalfängeraktivität, Gesamtphenol und 2,2-Azino-bis-(3-Ethylbenzothiazolin-6-sulfonat-Assay-Methode bestimmt. Die HPTLC-Studie wurde auf einer vorbeschichteten Kieselgel 60 F254 TLC-Platte durchgeführt, als mobile Phase wurde Toluol:Ethylacetat:Ameisensäure (5,5:4,5:1, v/v/v) verwendet und die UV-Detektion erfolgte bei 254 und 366 nm. Für die Derivatisierung wurde ein Anisaldehyd-Schwefelsäure-Reagenz verwendet. **Ergebnisse:** Das Ergebnis zeigte, dass der antioxidative Test der homöopathischen Urtinktur von *Hydrocotyle asiatica* eine herausragende antioxidative Aktivität aufwies. Die HPTLC-Studie zeigte das Vorhandensein der Triterpenglykosidverbindung Asiatische Säure im Chloroformextrakt von *Hydrocotyle asiatica*. **Schlussfolgerung:** Die aus authentifizierten Pflanzenproben hergestellten Urtinkturen wiesen im Vergleich zu den aus dem Handel bezogenen Urtinkturen ein Maximum an aktiven Bestandteilen und eine ausgeprägte antioxidative Aktivität auf. Die vorliegende Studie rechtfertigt die homöopathische Verwendung von *Hydrocotyle asiatica* und unterstreicht seine heilenden Eigenschaften.

## होम्योपैथी में इस्तेमाल होने वाले ऑक्सीकरण रोधी विशेषता से युक्त हाइड्रोकोटाईल एशियाटिका मूल अपमिश्रण तथा उच्च प्रदर्शनीय तनुस्तरिय वर्णलेखन के तुलनात्मक अध्ययन

**परिचय:** भारतीय दवा पद्धति में हाइड्रोकोटाईल एशियाटिका की उपचार महत्वता बहुत अधिक है क्योंकि इसमें ऑक्सीकरण रोधी क्षमता भरपूर मात्रा में होती है। होम्योपैथी में, हाइड्रोकोटाईल एशियाटिका का इस्तेमाल पीलिया, त्वचा रोगों, जलोदर, पीलपाँव, कुष्ठ रोग, प्रमेह, श्वेत प्रदर तथा तंत्रिका दुर्बलता का उपचार करने में किया जाता है। इसमें ट्राइटरपीन ग्लाइकोसाइड एशियाटिक एसिड की मात्रा सबसे प्रचुर होती है जो कैसर कोशिकाओं पर कोशिकीय विषाक्तता क्रियाशीलता दर्शाता है। इस दवा का मूल होम्योपैथिक अपमिश्रण ऑक्सीकरण रोधी यौगिकों का प्रमुख स्रोत है, जो कि समग्र औषधीय कार्यशीलता के लिए उत्तरदायी है। **विषय सारांश:** इस अध्ययन का उद्देश्य ऑक्सीकरण रोधी क्रियाशीलता, हाइड्रोकोटाईल एशियाटिका तथा उच्च तनुस्तरिय वर्णलेखन (एचपीटीएलसी) का आंकलन करना। **सामग्रियाँ एवं विधि:** इन हाउस मूल होम्योपैथी (ए) और तीन बाजार नमूनों (बी, सी और डी) की ऑक्सीकरण रोधी 2,2-डाई फिनाईल-1-पिक्रिल-हाइड्रोजिल मुक्त आयन हटाने की गतिविधि, कुल फिनाईलफिनुल और 2,2-एज़िनो-बिस-(3-एथिलबेंजोथियाज़ोलीन-6-सल्फोनेट परीक्षण विधियों द्वारा निर्धारित की गई थी। एचपीटीएलसी अध्ययन पूर्व-लेपित सिलिका जेल 60 एफ254 टीएलसी प्लेट पर किया गया था, का प्रयोग गतिमान अवस्था हेतु, टालुईन

एथिल एसीटेट: फॉर्मिक एसिड (5.5: 4.5: 1, आ / आ / आ) और पराबैंगनी परख 254 और 366 एनएम पर किया गया था। व्युत्पादन के लिए, एक एनिसाल्डिहाइड सल्फ्यूरिक एसिड अभिकर्मक का उपयोग किया गया था। **परिणाम:** परिणाम से पता चला कि हाइड्रोकोटाइल एशियाटिका के होम्योपैथिक मूल अर्क के ऑक्सीकरण रोधी परख में प्रमुख ऑक्सीकरण रोधी गतिविधि थी। एचपीटीएलसी अध्ययन ने हाइड्रोकोटाइल एशियाटिका के क्लोरोफॉर्म सार में ट्राइटरपीन ग्लाइकोसाइड यौगिक एशियाटिक एसिड की उपस्थिति का संकेत दिया। **निष्कर्ष:** प्रमाणीकृत पौधों के नमूनों द्वारा तैयार किए गए मूल अर्क ने बाजार से खरीदे गए मूलार्थ की तुलना में अधिकतम सक्रिय घटक और प्रमुख ऑक्सीकरण रोधी गतिविधि दिखाई। वर्तमान अध्ययन हाइड्रोकोटाइल एशियाटिका के होम्योपैथिक उपयोग को सही ठहराता है और इसके उपचार गुणों पर प्रकाश डालता है।

### Estudios comparativos de cromatografía en capa fina de alto rendimiento y potencial antioxidante de la tintura madre de *Hydrocotyle asiatica* utilizada en homeopatía

**Antecedentes:** *Hydrocotyle asiatica* tiene un gran significado terapéutico en el sistema indio de la medicina debido a su rica actividad antioxidante. En homeopatía, *Hydrocotyle asiatica* se utiliza para el tratamiento de la ictericia, enfermedades de la piel, hidropesía, elefantiasis, lepra, gonorrea, leucorrea y debilidad nerviosa. Contiene el glucósido triterpeno más abundante ácido asiático que muestra actividad citotóxica en las células cancerosas. La tintura madre homeopática de este fármaco es una fuente importante de compuestos antioxidantes, que es responsable de su actividad farmacológica general. **Objetivos:** El objetivo del estudio fue evaluar la actividad antioxidante y el estudio de cromatografía en capa fina de alto rendimiento (HPTLC) de tintura madre homeopática y muestras de mercado de *Hydrocotyle asiatica*. **Materiales y Métodos:** La actividad antioxidante de la tintura madre homeopática interna (A) y de tres muestras de mercado (B, C y D) se determinó mediante métodos de ensayo de 2,2-difenil-1-picril-hidrazil-hidrazil-actividad de depuración de radicales libres, fenol total y 2,2-azino-bis-(3-etilbenzotiazolina-6-sulfonato). Estudio realizado con HPTLC en placa de TLC de gel de sílice 60 F254 precubierta, la fase móvil utilizada fue tolueno:acetato de etilo:ácido fórmico (5,5:4,5:1, v/v/v) y detección UV a 254 y 366 nm. Para la derivación se utilizó un reactivo de ácido sulfúrico anisaldehído. **Resultados:** El resultado reveló que el análisis antioxidante de la tintura madre homeopática de *Hydrocotyle asiatica* tenía una actividad antioxidante prominente. El estudio de HPTLC indicó la presencia del compuesto glucósido triterpeno ácido asiático en el extracto de cloroformo de *Hydrocotyle asiatica*. **Conclusión:** Las tinturas madre preparadas por muestras vegetales autenticadas mostraron constituyentes activos máximos y actividad antioxidante prominente en comparación con las tinturas madre obtenidas del mercado. El presente estudio justifica el uso homeopático de *Hydrocotyle asiatica* y destaca sus propiedades curativas.

### [医]亚洲水样母酊用于顺势疗法的高效薄层色谱与抗氧化潜力的比较研究

**背景资料:** [医]亚洲水样由于其丰富的抗氧化活性,在印度医学体系中具有重要的治疗意义.在顺势疗法中,[医]亚洲水样用于治疗黄疸,皮肤病,水肿,象皮病,麻风病,淋病,白带和神经衰弱.它含有最丰富的三萜糖苷亚洲酸,对癌细胞显示细胞毒活性.这种药物的顺势疗法母酊是抗氧化化合物的主要来源,其对其整体药理活性负责. **目标:** 本研究的目的是评价抗氧化活性和高效薄层色谱(HPTLC)研究的[医]亚洲水样内部顺势疗法;顺势疗法母酊和市场样品. **材料和方法:** 采用2,2-二苯基-1-吡啶基-胍基-水合二氢自由基清除活性、总苯酚和2,2-叠氮基-双-(3-乙基苯并噻唑啉-6磺酸盐测定方法测定了内部同化母酊(A)和三个市场样品(B、C和D)的抗氧化活性.在预涂硅胶60F254TLC板上进行HPTLC研究,使用的流动相为甲苯:乙酸乙酯:甲酸(5.5:4.5:1, v/v/v),并在254和366nm处进行UV检测.为了衍生化,使用了茴香醛硫酸试剂. **结果:** 研究表明,亚洲水草顺势疗法母酊的抗氧化试验具有突出的抗氧化活性. HPTLC研究表明三萜苷化合物亚洲酸在[医]亚洲水样的氯仿提取物中存在. **结论:** 与从市场上采购的母酊相比,经认证的植物样品制备的母酊显示出最大的活性成分和突出的抗氧化活性. 本研究证明了亚洲氢钾的顺势疗法的使用,并强调了其治疗作用.