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HPTLC finger printing of aqueous root extract of *Rotula aquatica* for phenol, tannin and saponin contents

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ABSTRACT

Herbals are endowed with phytochemicals that have applications in different pharmaceutical formulations. There is development in extracting, identifying and quantifying these compounds that can lead to drug discovery. Various technological advancements are available for isolation and identification of these compounds. Rotula aquatica is a herbal plant used for the treatment of urolithiasis. The aqueous extract of plant root was used in the present study to identify few compounds that are present in the plant. Preliminary phytochemical screening showed the presence or absence of alkaloids, flavonoids, phenols, steroids, saponins, terpenoids, tannins, anthraquinones, anthocyanin, quinones, volatile oils, proteins and carbohydrates. HPTLC of aqueous extract of Rotula aquatica was carried out to assess the further assess the presence of phenol, tannin and saponins. The study revealed the presence of seven different types of phenols with seven different R_f values, twelve different types of tannins with twelve different R_f values and eleven different types of saponins with eleven different R_f values. These compounds identification can be used as a tool for identification of the plant and utilisation in the medicine formulation.

Key words: *Rotula aquatica*, phenol, tannin, saponin, HPTLC

INTRODUCTION

Indian traditional healthcare system uses many medicinal plants that are known for their therapeutic action. Some of these plants are thoroughly investigated and few are under investigation for their active ingredients. Since the available information in the pharmacopoeia are physico chemical parameters but the standardization of the drugs is need for the day. The modern methods of identification and quantification of the active constituents in the plant materials are useful for the standardization and formulation of herbal medicines.

Herbal medicines have several phytoconstituent and they exert beneficial effect on human tissue. Drugs with multiple mechanism of protection are one way to reduce injury to human tissues. In India Ayurvedic system of medicine utilizes te principle of pashana bheda to treat urinary stones. Herbal medicines derived from plant extracts are utilized to treat a variety of ailments. There is relatively little knowledge regarding their mode of action. There is an increasing interest and acceptance towards the herbal medicines. They safeguard naturally against development of certain conditions and be a putative treatment for some diseases[1]. HPTLC is a technique that offers the resolution and estimation of active constituents in the plant. It can also be used for the identification of the plant as a

phytochemical marker and estimator of genetic variability in plant populations. It is proved as a linear, precise, accurate method for the identification and authentication and characterization of medicinally important plant. HPTLC will help the manufacturer for quality control and standardization of herbal formulations. It is tool for the evaluation of botanical materials that allows the analysis of a broad number of compounds both efficiently and cost effectively [2].

The plant *Rotula aquatica* commonly called as pashan bed belonging to the family boraginaceae is widely distributed in India from Kumaon to Assam and western to southern India. The plant is used for its antiurolithiatic activity. It has active ingredients in the orm of phytochemicals such as alkaloids, flavanoids, phenolic compounds and proteins and amino acids that produce a definite action on the human body [3].

In the present study, *Rotula aquatica* plant was used. The plant parts were used for different analysis of the active ingredients. The petroleum ether, chloroform, methanol and aqueous root extracts were prepared and preliminary phytochemical assay was conducted. Further based on the preliminary screening results, the aqueous extract was used for the HPTLC analysis of phenol, tannin and saponin contents.

MATERIALS AND METHODS

Plant Material

The fresh plant of *Rotula aquatica* Lour. was collected from Kuttiyadi (Malapuram district) in Kerala State, India. The collected plant material was identified and their authenticity was confirmed by comparing the voucher specimen at the herbarium of Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu. The herbarium registered number was BSI/SRC/5/23/2012-13/Tech/415.

Processing of the plant material

Freshly collected plant material was cleaned to remove adhering dust, divided into different parts (leaf, stem, root, flower and fruit) and then dried under shade. The dried samples were powdered and used for further studies

Successive solvent extraction

The air dried, powdered plant material was extracted in Soxhlet extractor successively with petroleum ether, chloroform, methanol and aqueous. Each time before extracting with the next solvent, the material was dried in hot air oven. Finally, the material was macerated using hot water with occasional stirring for 24 hrs and the aqueous extract was filtered. The different solvent extracts were concentrated by rotary vacuum evaporator and then air dried. The dried extract obtained with each solvent was weighed.

High Performance Thin Layer Chromatography (HPTLC)

A Camag HPTLC system consisting of a Linomat V applicator, TLC scanner 3, Reprostar 3 with 12 bit CCD camera for photo documentation, controlled by WinCATS-4 software was used for the analysis of aqueous root extract. All the solvents used for HPTLC analysis were obtained from MERCK. 100mg sample was dissolved in 1ml of HPTLC Grade water and centrifuged at 3000rpm for 5 min and used for HPTLC analysis as test solution. The samples (2µl) were spotted in the form of bands of length 6mm with a Camag microlitre syringe on precoated silica gel glass plate 60F-254 (20cm×10cm) with 250µm thickness (E-Merck, Darmstadt, Germany) using a Camag Linomat IV (Switzerland). The sample loaded plate was kept in thin-layer chromatography (TLC) twin through developing chamber after saturated with solvent vapour with respective mobile phase and the plate was developed in the respective mobile phase up to 90 mm. Linear ascending development was carried out in (20 cm ×10 cm) twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with the mobile phase and the chromatoplate was developed twice with the same mobile phase to get good resolution of phytochemical contents. The optimized chamber saturation time for mobile phase was 30 min at room temperature [(25±2) ºC]. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under white light, UV light at 254 and 366 nm. The plate was photo-documented at UV 366 nm and daylight using photodocumentation (CAMAG REPROSTAR 3) chamber. Finally, the plate was fixed in scanner stage and scanning was done at 366 nm. The Rf values and finger print data were recorded by WIN CATS software (Version 1.3.4, Camag).

Phenol
Toluene-Acetone-Formic acid (4.5: 4.5: 1) was employed as mobile phase. The plate was sprayed with 20% sodium carbonate solution followed by Folin Ciocalteu reagent and dried at 100 ºC in hot air oven for 3 min.

Saponin
Chloroform-Glacial acetic acid-Methanol-Water (6.4: 3.2: 1.2: 0.8) was employed as mobile phase. The plate was sprayed with anisaldehyde sulphuric acid reagent and dried at 100 ºC in hot air oven for 3 min.

Tannin
Toluene-Ethyl acetate-Formic acid-Methanol (3: 3: 0.8: 0.2) was employed as mobile phase. The plate was sprayed with 5% ferric chloride reagent and dried at 100 ºC in hot air oven for 3 min.

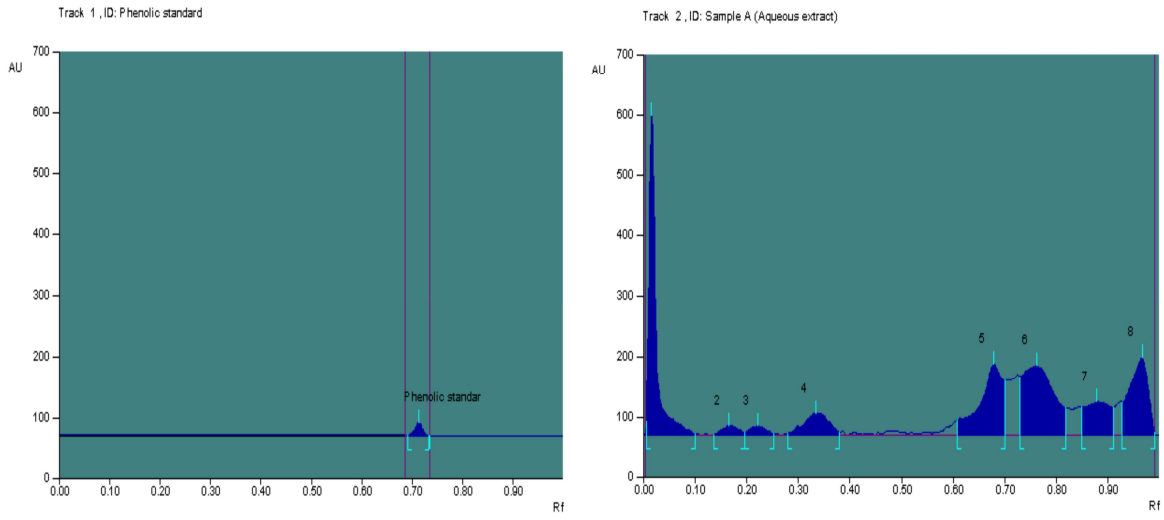
RESULTS AND DISCUSSION

Phenol profile
HPTLC analysis of aqueous root extract of *R. aquatica* with Toluene-Acetone-Formic acid (4.5: 4.5: 1) showed the presence of seven different types of phenols with seven different R_f values that ranged from 0.01 to 0.97 (Table 1) compared to other mobile phases. The phenol profile of aqueous extract of *R. aquatica* root is depicted in Fig 1,2 and compared with HPTLC chromatogram of standard kaempferol. The comparison of R_f values of sample A with that of the standard kaempferol indicated that the aqueous root extract of *Rotula aquatica* may contain kaempferol.

Table 1. Phenol profile of the aqueous root extract of *R. aquatica*

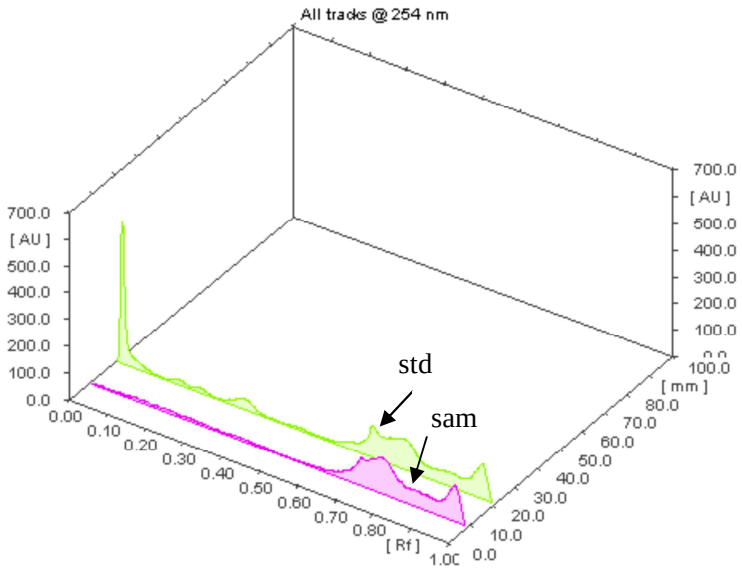
Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.71	98.4	3013.6	Phenolic standard
Sample A	1	0.01	474.5	4752.5	Unknown
Sample A	2	0.17	16.3	460.6	Unknown
Sample A	3	0.22	15.2	447.1	Unknown
Sample A	4	0.33	36.7	1659.6	Unknown
Sample A	5	0.68	115.9	4604.4	Phenolic 1
Sample A	6	0.76	111.9	6177.1	Phenolic 2
Sample A	7	0.88	51.5	2305.9	Unknown
Sample A	8	0.97	123.3	3980.6	Unknown

Figure 1. Densitogram display of aqueous root extract of *R. aquatica* for detection of phenol compounds



a) HPTLC chromatogram of standard kaempferol – peak densitogram display (scanned at 366nm); b) HPTLC chromatogram of aqueous root extract of R. aquatica – peak densitogram display (scanned at 366nm).

Figure 2. 3D display of HPTLC chromatogram of aqueous root extract of *R. aquatica*



The phenolic compounds exert multiple biological effects like antioxidant, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic activity. The aqueous extract of *Spermacoce hispida* showed the presence of five different types of phenols with five different R_f values with range of 0.03 to 0.78[4]. The experiment by [5] also revealed the presence of three different types of phenol with three different types of R_f value ranging from 0.63 to 0.95 in *Costus speciosus* rhizome. [6] found that the methanol root extract of *Pandanus odoratissimus* contained five different types of phenols with five different R_f value with a range of 0.24 to 0.70. [7] has observed that *Aspalathus linearis* have 25 and 30 phenolic compounds in aqueous and ethanolic extracts. Similar to the present study, [8] also has reported the presence of phenolic compounds in the root extract of watercress.

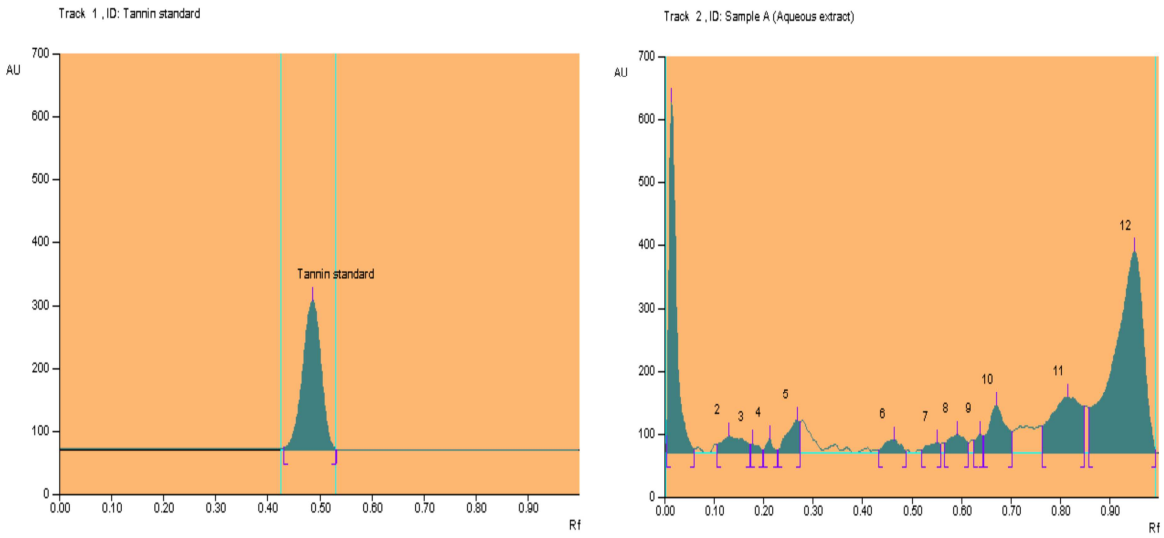
Tannin profile

Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. Toluene-Ethyl acetate-Formic acid-Methanol (3:3:0.8:0.2) showed the presence of twelve different types of tannins with twelve different R_f values with a range of 0.01 to 0.95 (Table 2). The tannin profile of *R. aquatica* is compared with HPTLC chromatogram of standard gallic acid which is illustrated in Fig 3 and 4.

Table 2. Tannin profile of the aqueous root extract of *R. aquatic*

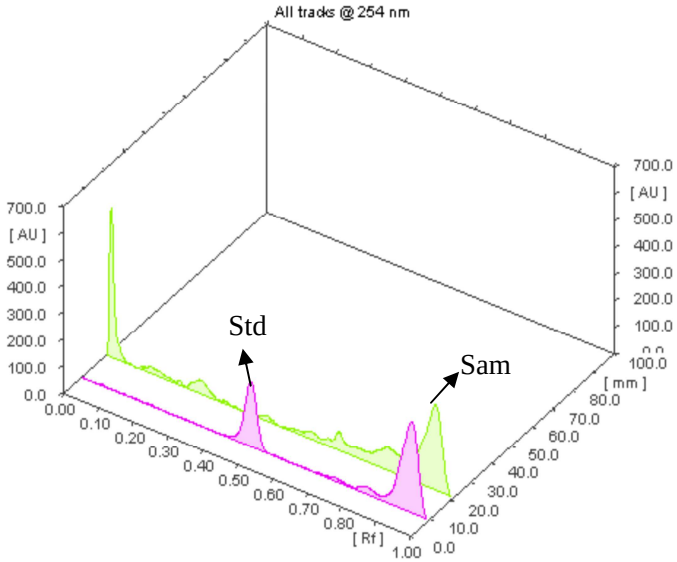
Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.49	241.7	6689.1	Tannin standard
Sample A	1	0.01	534.6	6689.1	Unknown
Sample A	2	0.13	26.3	1092.3	Unknown
Sample A	3	0.18	14.7	206.4	Unknown
Sample A	4	0.21	21.9	261.5	Unknown
Sample A	5	0.27	50.9	1152.9	Unknown
Sample A	6	0.46	20.8	597.3	Tannin 1
Sample A	7	0.55	15.9	1152.9	Unknown
Sample A	8	0.59	29.3	908.6	Unknown
Sample A	9	0.64	29.4	417.7	Unknown
Sample A	10	0.67	74.5	2272.5	Unknown
Sample A	11	0.81	87.9	4884.6	Unknown
Sample A	12	0.95	319.3	17137.0	Unknown

Figure 3. Densitogram display of aqueous root extract of *R. aquatica* for detection of tannin compounds



a) HPTLC chromatogram of standard gallic acid – peak densitogram display (scanned at 366nm); b) HPTLC chromatogram of aqueous root extract of R. aquatica – peak densitogram display (scanned at 366nm).

Figure 4. 3D display of HPTLC chromatogram of aqueous root extract of *R. aquatica*

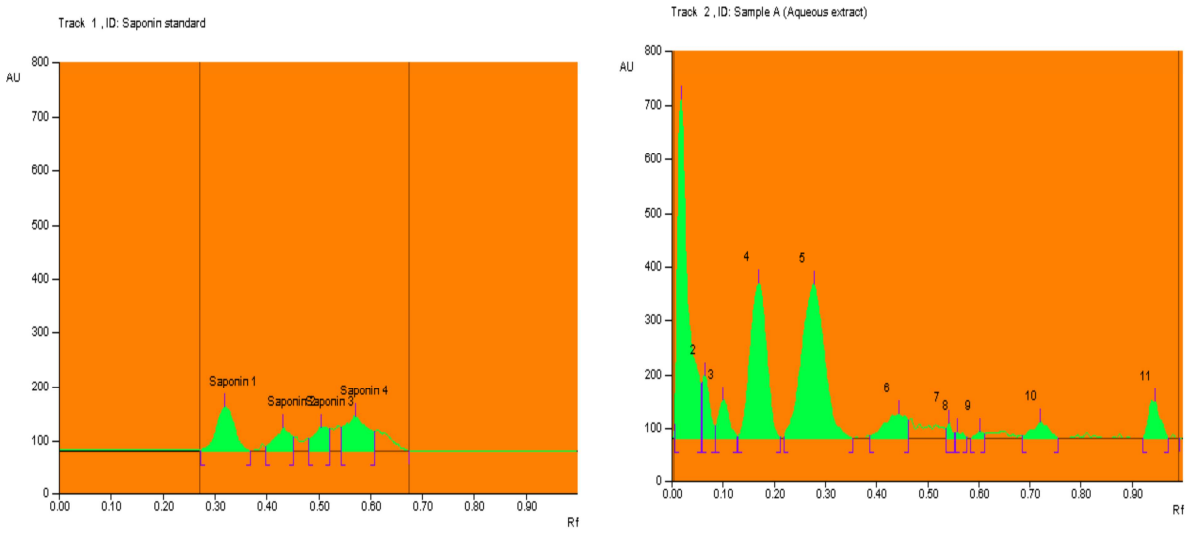


The present result coincides with the findings of [9] where the ethanolic extract of *Maranta arundinacea* rhizome showed the presence of thirteen different types of tannin with thirteen different R_f values in the range of 0.48 to 0.93. As in the present study, [10] also revealed the presence of twenty different types of tannin with twenty different R_f values with a range of 0.06 to 0.79 and 0.03 to 0.86 respectively in the aqueous extract of *Passiflora edulis* and *Bauhinia tomentosa*. The experiment conducted with the methanolic leaf extract of *Annona squamosa* showed the presence of eleven different types of tannins with eleven different R_f values with range from 0.05 to 0.83[11].

Saponin profile

Chloroform-Glacial acetic acid-Methanol-Water (6.4: 3.2: 1.2: 0.8) mobile phase was used for HPTLC studies of aqueous root extract of *R. aquatica*. The study revealed the presence of eleven different types of saponins with eleven different R_f values ranging from 0.02 to 0.94 (Fig 5 and 6, Table 3). The saponin profile of *R. aquatica* was compared with HPTLC chromatogram of standard saponin.

Figure 5. Densitogram display of aqueous root extract of *R. aquatica* for detection of saponin compounds



a) HPTLC chromatogram of standard saponin – peak densitogram display (scanned at 366nm); b) HPTLC chromatogram of aqueous root extract of R. aquatica – peak densitogram display (scanned at 366nm).

Figure 6. 3D display of HPTLC chromatogram of aqueous root extract of *R. aquatica*

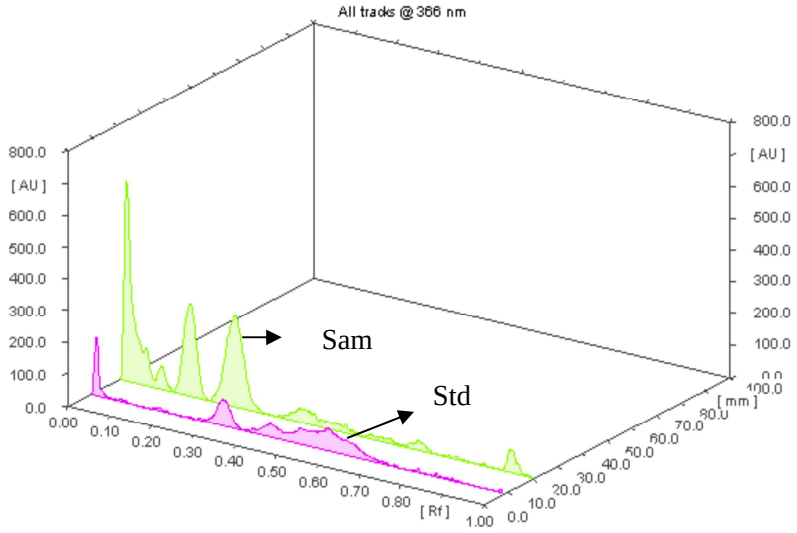


Table 3. Saponin profile of the aqueous root extract of *R. aquatic*

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.32	83.6	2800.1	Saponin 1
STD	2	0.43	46.5	1326.9	Saponin 2
STD	3	0.50	50.0	1328.9	Saponin 3
STD	4	0.57	72.2	2929.2	Saponin 4
Sample A	1	0.02	629.2	11665.2	Unknown
Sample A	2	0.06	118.0	1564.5	Unknown
Sample A	3	0.10	70.4	1337.3	Unknown
Sample A	4	0.17	288.4	8370.7	Unknown
Sample A	5	0.28	286.2	11599.3	Saponin 1
Sample A	6	0.44	44.7	1765.7	Saponin 2
Sample A	7	0.54	27.7	266.0	Saponin 3
Sample A	8	0.56	11.7	152.3	Saponin 4
Sample A	9	0.60	12.5	177.1	Saponin 5
Sample A	10	0.72	31.6	864.2	Unknown
Sample A	11	0.94	69.2	1426.3	Unknown

The results of the present study are in agreement with [12] who recorded that the methanolic extract of stem, leaves, root, flower and seeds of *Aerva lanata* showed the presence of twenty one different types of saponins with twenty one different R_f values with a range of 0.01 to 0.98. [13] also reported that the ethanol leaf extract of *Eugenia floccosa* showed the presence of five different types of saponins with five different R_f values with a range of 0.08 to 0.96. [14] revealed that the leaf of *Morinda pubescens* showed the presence of nine different types of saponins with nine different R_f values with a range of 0.07 to 0.66. [15] reported that the methanol leaf extract of *Barleria cristata* contained eleven different types of saponins with eleven different R_f values with range from 0.02 to 0.78. [16] based on the fingerprint profile of *Ficus nervosa* recommends it to be a effective diagnostic tool for correct identification of the plant and its active ingredients.

CONCLUSION

The medicinal values of a plant lie in their phytochemical components. The HPTLC analysis was carried out to assess the chemical constituent's profile of plants with botanical identity and estimation of biochemical markers. The aqueous extract was studied with different composition of mobile phases and suitable ones were observed. The HPTLC analysis confirmed the presence of phenols, tannins and saponins.

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