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Original Article

Exploring the Logic Behind the Usage of Fresh Drugs in Ayurvedic Formulations through Preliminary Phytochemistry and HPTLC Analysis

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ABSTRACT

Background: Ayurvedic principles provide guidelines for medicinal herb usage, emphasizing differences in fresh and dried forms. Emphasis is given to the use of doubled quantities of raw drugs when taken in fresh form, focusing on some exceptions like *Guduchi* (*Tinospora cordifolia* Linn.), where double quantity is not required even if taken in fresh form. Despite this, practical challenges often favour the prevalent use of dried forms. **Objectives:** To validate the Ayurvedic principle for drug selection in formulations through preliminary phytochemistry and HPTLC analysis of *Tinospora cordifolia* Linn., stems and the decoction prepared with fresh and dry samples in different amounts. **Materials and Methods:** Fresh and dried *Tinospora cordifolia* Linn., stems were separately extracted with hexane, chloroform, ethyl acetate and methanol using a Soxhlet continuous extraction apparatus. Guduchi decoctions were prepared with dry and fresh drugs in the required quantities and double quantities. Chromatographic studies of these samples were analyzed using HPTLC with specific mobile phases. **Results:** Preliminary physicochemical analysis provided similar results for fresh and dry samples. HPTLC analysis revealed the presence of more spots in decoction prepared with new samples and the spots were practically identical irrespective of the quantity taken. Analysis of dry Guduchi samples suggests that taking double the required amount in dry form is better. Further, the methanol extracts exhibited more spots, indicating the prevalence of polar compounds. **Conclusion:** This study enhances our understanding of Ayurvedic drug usage, suggesting using fresh Guduchi samples in decoctions. For dry samples, doubling the quantity is advised for optimal efficacy. It also provides a foundation for further exploration of its medicinal efficacy.

Keywords: Fresh and Dry drug selection, Guduchi, HPTLC, Preliminary phytochemistry,Soxlet extraction, *Tinospora cordifolia*.

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INTRODUCTION

Plants are natural assets and plant products are gaining global importance in the healthcare sector. Over 80% of the global population relies on natural products, while approximately 65% of the Indian population depends on traditional medicine systems.[1] Herbal medications are an essential component of many Ayurvedic prescription formulas. Ayurvedic principles provide guidelines for medicinal herb collection and selection in formulations. According to Bhaishajya Ratnavali[2] As a general rule, fresh raw drugs are to be chosen in double the quantity required, except for some specific drugs required; these exceptional drugs are to be taken mandatorily in fresh form, but double quantity is not needed. Eg: *Guduchi* (*Tinospora cordifolia* Linn.), *Kutaja* (*Holarrhena antidysentrica* Linn.), *Vasa* (Adhatoda vasica Nees.), *Shatavari* (*Asparagus racemosus* Willd.), etc.,[3] But in the era of urbanization and medicinal plant scarcity, it may only sometimes become practical to use raw drugs in fresh form while preparing formulations, prompting the manufacturers to meet the needs with dried raw drugs. The efficacy of the formulations when prepared using fresh and dry raw drugs may be different and needs to be fully understood. It requires some scientific evidence to conclude if dry drugs can meet the requirement. The quality and efficacy of formulations prepared using fresh and dry raw drugs differ. In that case, it may further become better to avoid the current trend of using dry drugs instead of fresh medicines in the case of the previously mentioned exceptional drugs, where it is mandatory to use fresh raw drugs. This study has been planned as a preliminary step to get further evidence so that future studies

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can be adequately designed to check the efficacy when prepared using dry and fresh forms. Preliminary phytochemistry and HPTLC have been done in this study to get any clues so that more extensive studies can be planned to explore the logic behind the form of drug selection and promote the usage of drugs in the required form. There is similar scientific evidence that *Shatavari Ghrita* made with the same amount of wet *Shatavari* (*Asparagus racemosus* Willd.) showed a higher percentage of Shatavarin IV than that of a dry sample. This means the fresh form should be used instead of doubling the amount, which aligns with the wet and dry drug collection principle.[4]

MATERIALS AND METHODS

Plant Collection

The fresh and dried stems of *Tinospora cordifolia* samples were collected from different parts of Cheruthuruthy, Kerala, India. All chemical solvents and reagents used in the qualitative and quantitative analysis were of analytical grade and lab grade, procured from Nice, Sigma-Aldrich and Merck Chemicals.

Extraction of plant material

The fresh and dried plant materials (100 g) were comprehensively extracted separately with 150 mL each of hexane, chloroform, ethyl acetate and methanol, respectively, using a Soxhlet extraction apparatus.[5]

Preparation of Guduchi decoction-methanol extract

Fresh and dried Guduchi samples of 250 g each were weighed accurately, cleaned with water and crushed using a grinder. The decoction was prepared with 250 g of samples as as per Sarngadhara Samhita's reference.[6] Hence, 250 g each of fresh and dry samples of Guduchi were taken separately, boiled four times (i.e., 1000 mL) of water in separate vessels and reduced to 1/4th quantity, i.e., 250 mL. As per the general guidelines, if fresh drugs were taken as raw materials for the preparation of formulations, it is to be taken in double quantity and since Guduchi is an exceptional drug in which double quantity is not required even if taken in fresh forms, the decoction was also prepared with a double quantity of fresh and dry samples of Guduchi. This was done to check the difference in preliminary phytochemistry and HPTLC to find out whether there is any difference between the formulations prepared using fresh and dry samples of Guduchi in the required quantity and double quantity. Hence, double quantity, i.e. 500 g each of fresh and dry samples of Guduchi, were also taken separately, boiled in the same quantity (i.e., 1000 mL) of water in separate vessels and reduced to 1/4th quantity, i.e., 250 mL. These decoctions were further filtered and evaporated on a water bath to remove water; the dried mass was refluxed with methanol and filtered.

Chromatographic studies

For the HPTLC analysis, all the Guduchi samples are prepared at 25 mg/mL concentration by dissolving plant extracts in HPLC-grade methanol as a solvent. Sonicated for 15 min at 40°C and filtered through the 0.22 µ PTFE filter paper and samples stored in refrigerator further process. The HPTLC plate pre-coated with silica gel 60 F_{254} thicknesses 0.2 mm was used as the stationary phase. Ethyl acetate: formic acid: acetic acid: water (10:1.1:1.1:2.3 v/v) were used as the mobile phase. Samples were spotted in triplicates of 5 µL each sample using Linomate 5 on the TLC plate and dried for a few minutes. Then, plates were kept in a TLC chamber containing the mobile phase and allowed to run up to three-fourths of the plate. After development, the plate was dried at 105°C and examined with the help of a visualizer. The R*f* values were observed using WinCATS software.[7]

Qualitative chemical examinations of extracts (Preliminary phytochemical evaluation)[8,9]

Different plant extracts were subjected to various chemical tests to detect chemical constituents present in them.

Detection of Alkaloids: Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used for detecting alkaloids by tests such as Mayer's, Wagner's, Dragendorff's and Hager's tests.

Detection of Flavonoids: The presence of flavonoids was detected by different tests such as alkaline reagent test, Lead acetate test and Zinc hydrochloric acid reduction test.

Detection of Carbohydrates: Extracts were dissolved individually in distilled water and filtered. The filtrates were used for detecting carbohydrates by different tests, such as the Molisch test, Benedict test and Fehling's test.

Detection of Glycosides: Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used for detecting alkaloids by different tests, such as Modified Borntrager's and Legal's tests.

Detection of Saponins: Froth test is used for the detection of saponins. Extracts were treated with 20 mL of distilled water and shaken vigorously in a graduated cylinder for 15 min. The formation of foam at a height of 1cm indicates the presence of saponins.

Detection of Fixed Oils and Fats: A stain test is used to identify fixed oil. The formation of greasy stains on filter paper indicates the presence of fixed oils and fats.

Detection of Phenols: It is done by a ferric chloride test. Extracts were treated individually with a few drops of ferric chloride solution. The formation of a bluish-black colour indicates the presence of phenols.

Detection of Tannin: It is done by a gelatin test. The formation of white precipitates indicates the presence of tannins.

Detection of Protein and Amino Acids: The presence of amino acids, proteins, peptides and aromatic amino acids was detected by Xanthoprotic and Ninhydrin tests.

Detection of Resins: Acetone water test is used to detect resins. The formation of turbidity indicates the presence of resins.

Detection of Diterpenes: It is conducted by copper acetate test. The appearance of emerald colour indicates the presence of diterpenes.

Detection of Phytosterols: Salkowski's test is used to detect phytosterols. The appearance of a golden yellow colour indicates the presence of triterpenes.

RESULTS

Preliminary phytochemical analysis of hexane, chloroform, ethyl acetate and methanol extracts of fresh and dry *Tinospora cordifolia* Linn., samples were almost similar, as mentioned in Table 1. Further, the same chemical analysis of the methanolic extract of decoction prepared using fresh and dry samples of *Tinospora cordifolia* Linn. in 250 g and 500 g quantities is given in Table 2. However, methanolic extracts of decoction prepared with 250 g of fresh and dry samples and 500 g of fresh and dry samples of *Tinospora cordifolia* Linn. showed similar Qualitative chemical analysis results. Furthermore, 08 spots were observed and mentioned in Figure 1 at 366 nm and 254 nm for the methanolic extract of decoction prepared with 250 g fresh Guduchi sample. And 08 spots also appeared and were mentioned in Figure 2 at 254 and 366 nm, methanolic extract of decoction prepared with 500 g of a fresh sample.

The R_f values @ 254 and 366 nm were compared in Table 3 of both methanolic extracts of decoction prepared with 250 g and 500 g of Guduchi samples in the same quantity of water. The results reveal that the spots are almost similar in this case; we can assume that there may not be a marked difference in the decoction even if the drug is not taken in double quantity if used in fresh form in the decoction.

However, in the case of the methanolic extract of decoction prepared with 250 g dry Guduchi samples HPTLC Chromatograms, 04 and 03 spots appeared, respectively, at 254 nm and 366 nm, as mentioned in Figure 3. The same dry Guduchi sample of 500 g of methanolic extract of decoction Figure 4 showed 08 spots in HPTLC Chromatograms at 254 and 366 nm, respectively. Further, the comparison of R_f values at 254 nm and 366 nm is given in Table 3 of the methanolic extract of decoction prepared with 250 g and 500 g of dry Guduchi samples. The spots obtained in the methanolic extract of decoction prepared with 500 g of dry Guduchi sample were high (8 spots in both 254 nm and 366 nm) when compared to that prepared with 250 g of dry

Table 1: Preliminary phytochemical analysis of different extracts of *Fresh* and *Dry* samples of *Tinospora cordifolia* Linn.

Sl. No.	Name of the Phytochemicals	Name of the Test	Guduchi Samples Extracts							
			Hexane Extract		Chloroform Extract		Ethyl acetate Extract		Methanolic Extract	
			Fresh	*Dry*	*Fresh*	*Dry*	*Fresh*	*Dry*	*Fresh*	*Dry*
1	Alkaloids	Dragendorff's	+	+	+	+	+	+	+	+
2	Carbohydrates	Fehling's test	-	+	-	+	+	-	+	+
3	Glycosides	Modified Borntrager's test	-	-	-	-	-	-	-	-
		Legal's test	-	-	-	+	+	-	+	+
4	Saponins	Froth test	-	+	+	+	+	+	+	+
5	Phytosterol	Salkowski's test	-	+	-	+	+	+	-	+
6	Resins	Acetone-water test	+	+	+	+	+	+	+	+
7	Phenols	Ferric chloride	+	+	+	+	+	+	+	+
8	Fixed oil	Stain test	+	+	-	-	+	+	+	+
9	Tannins	Gelatin test	+	-	+	-	-	-	+	+
10	Protein and amino acid	Xanthoprotectic test	-	-	-	-	-	+	+	+
		Ninhydrin test	+	-	-	-	+	-	+	-
11	Flavonoids	Alkaline reagent test	+	-	+	-	+	-	+	-
		Lead acetate test	-	-	+	-	-	-	+	-

Note: + Present, - Absent.

Table 2: Preliminary phytochemical analysis of methanolic extract of decoction prepared using *Fresh* and *Dry* samples of *Tinospora cordifolia* Linn. in 250 g and 500 g quantity.

Sl. No.	Name of the Phytochemical	Name of the Test	Different quantity of Guduchi Samples			
			Fresh Sample-Methanolic extract		*Dry Sample-Methanolic extract*	
			250 g	500 g	250 g	500 g
1	Alkaloids	Dragendorff's	+	+	+	+
2	Carbohydrates	Fehling's test	+	+	+	+
3	Glycosides	Modified Borntrager's test	-	-	-	-
		Legal's test	+	+	+	+
4	Saponins	Froth test	+	+	+	+
5	Phytosterol	Salkowski's test	+	+	+	+
6	Resins	Acetone-water test	+	+	+	+
7	Phenols	Ferric chloride	+	+	+	+
8	Fixed oil	Stain test	+	+	+	+
9	Tannins	Gelatin test	+	+	+	+
10	Protein and amino acid	Xanthoprotectic test	+	+	+	+
		Ninhydrin test	+	+	+	+
11	Flavonoids	Alkaline reagent test	+	+	+	+
		Lead acetate test	+	+	+	+

Note: + Present, - Absent.

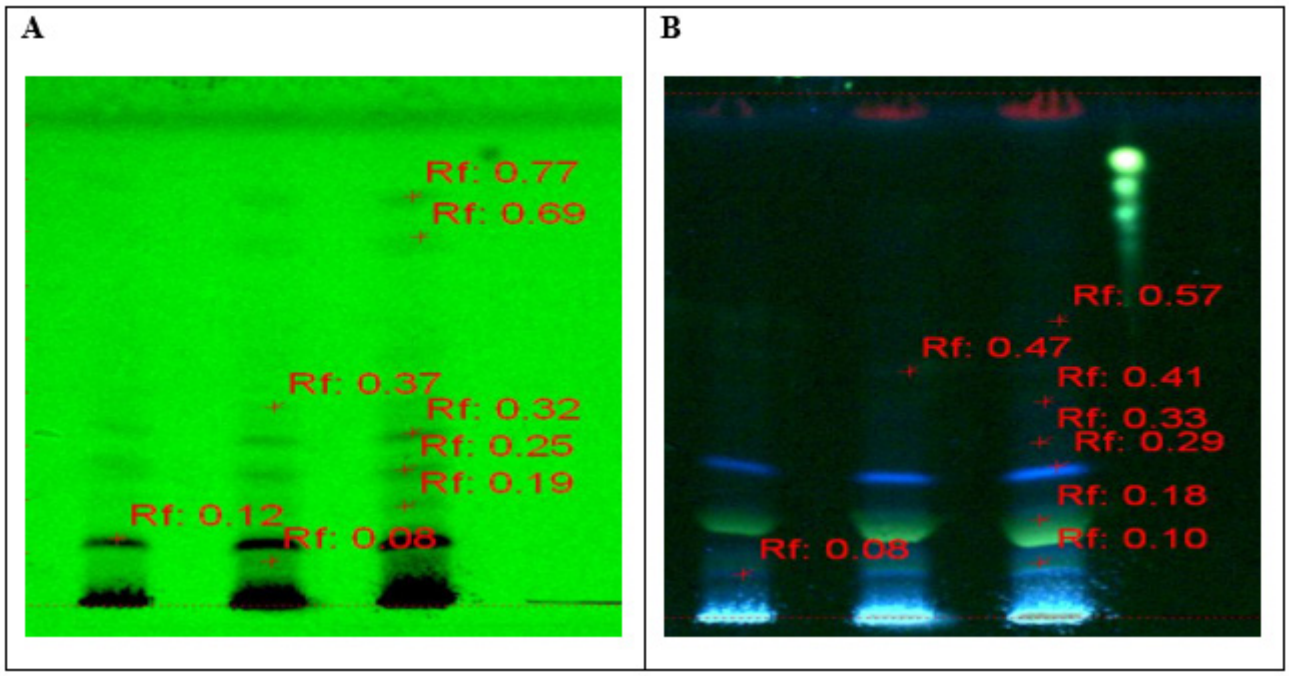

Figure 1: HPTLC Chromatograms of decoction prepared using 250 g of *Fresh* sample of *Tinospora cordifolia* Linn. stems {@ 254 (A) and 366 nm (B)}.

Guduchi sample (four and three spots, ethyl acetate, chloroform and methanol extracts at 254 and 366 nm, respectively, at 254 nm and 366 nm).

This study also observed the different solvent effects in extracting dry and fresh Guduchi samples in 250 g and 500 g quantities, respectively. Soxhlet extraction of hexane, ethyl acetate, chloroform and methanol solvent is used for the above samples. Figure 5 shows the chromatogram HPTLC of fresh samples of *Tinospora cordifolia* Linn. stems**,** ethyl acetate, chloroform and methanol extracts at 254 and 366 nm, respectively. Where the

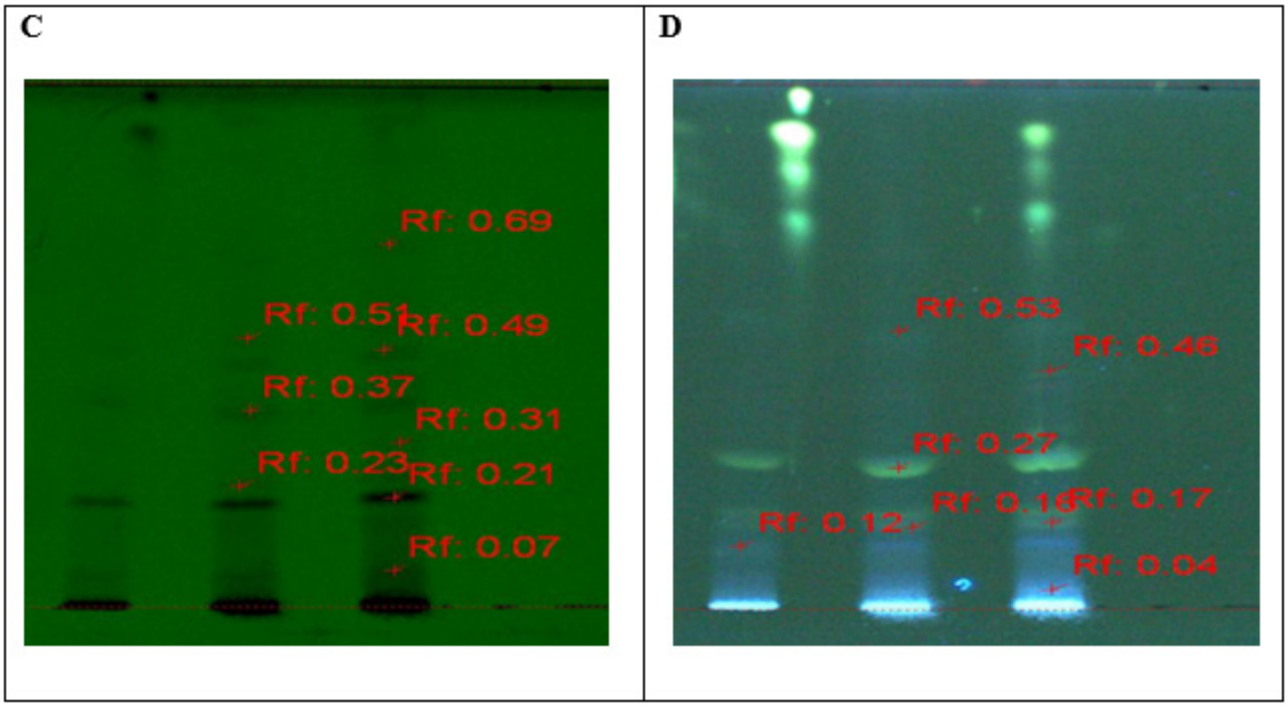

Figure 2: HPTLC Chromatograms of decoction prepared using 500 g of *Fresh* sample of *Tinospora cordifolia* Linn. stems {@ 254 (C) and 366 nm (D)}.

Table 3: Comparable R_f values in the methanolic extract of decoction prepared with 250 g and 500 g of *Dry* and *Fresh* Guduchi sample at 254 and 366 nm.

Sl. No.	Name of the Sample	R_f* values @ 254 nm**		R_f values @ 366 nm	
		250 g	500 g	250 g	500 g
1	Methanolic extract of *Dry* Guduchi Sample	04-Spots: 0.09, 0.27, 0.40, 0.55	08-Spots: 0.04, 0.07, 0.12, 0.18, 0.25, 0.29 0.38, 0.40	03-Spots: 0.06, 0.13 0.39	08-Spots: 0.05,0.09 0.17, 0.27 0.35, 0.39 0.53, 0.73
2	Methanolic extract of *Fresh* Guduchi Sample	08-Spots: 0.08, 0.12, 0.19, 0.25 0.32, 0.37 0.69, 0.77	08-Spots: 0.07, 0.21, 0.23, 0.31, 0.37, 0.49, 0.51, 0.69	08-Spots: 0.08, 0.10, 0.18, 0.29, 0.33, 0.41, 0.47, 0.57	07-Spots: 0.04, 0.12, 0.16, 0.17, 0.27, 0.46, 0.53

*Retardation factor, ** Nano metre.

dry samples of *Tinospora cordifolia* Linn. stems, ethyl acetate, chloroform and methanol extracts at 254 and 366 nm, respectively, are shown in Figure 6. Further, R_f values comparison of 250 and 500 g dry and fresh Guduchi samples of different extract hexane to methanol and decoction prepared with required and double quantity of stems are mentioned in Table 4.

DISCUSSION

Ayurvedic principles provide guidelines for medicinal herb usage, emphasizing differences in the selection of fresh and dried forms in formulations. Special importance is given to the use of doubled quantities of raw drugs than required when taken in fresh form, focusing on some exceptions like *Guduchi* (*Tinospora cordifolia* Linn.). For these exceptional drugs, a double quantity is not required, even if taken in fresh form. Despite this, practical challenges often favour the prevalent use of dried forms. This study has been done to validate the principle of drug collection in fresh and dry forms. Here, preliminary phytochemistry and HPTLC results of fresh and dry samples of Guduchi and the decoction prepared with the required quantity (250 g) and double quantity (500 g) of fresh samples were analyzed and compared. Also, efforts were made to compare the results of decoction prepared with dry samples in the required and double quantities to find the differences since it is prevalently used in dry form.

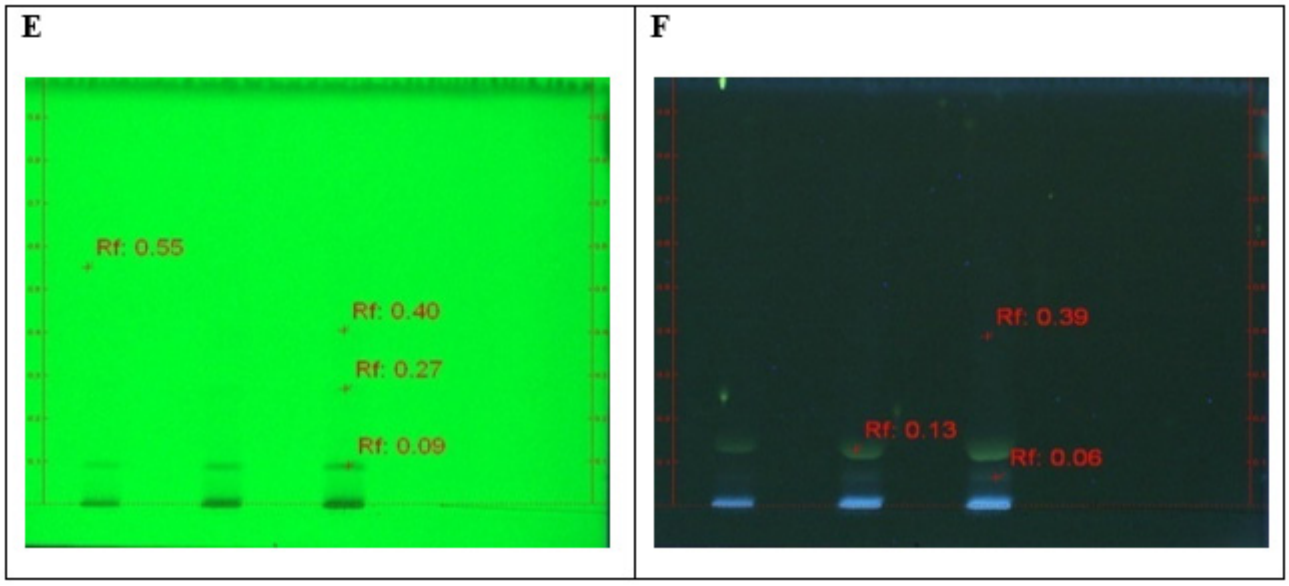

Figure 3: HPTLC Chromatograms of decoction prepared with 250g of *Dry* sample of *Tinospora cordifolia* Linn. stems {@ 254 (E) and 366 nm (F)}.

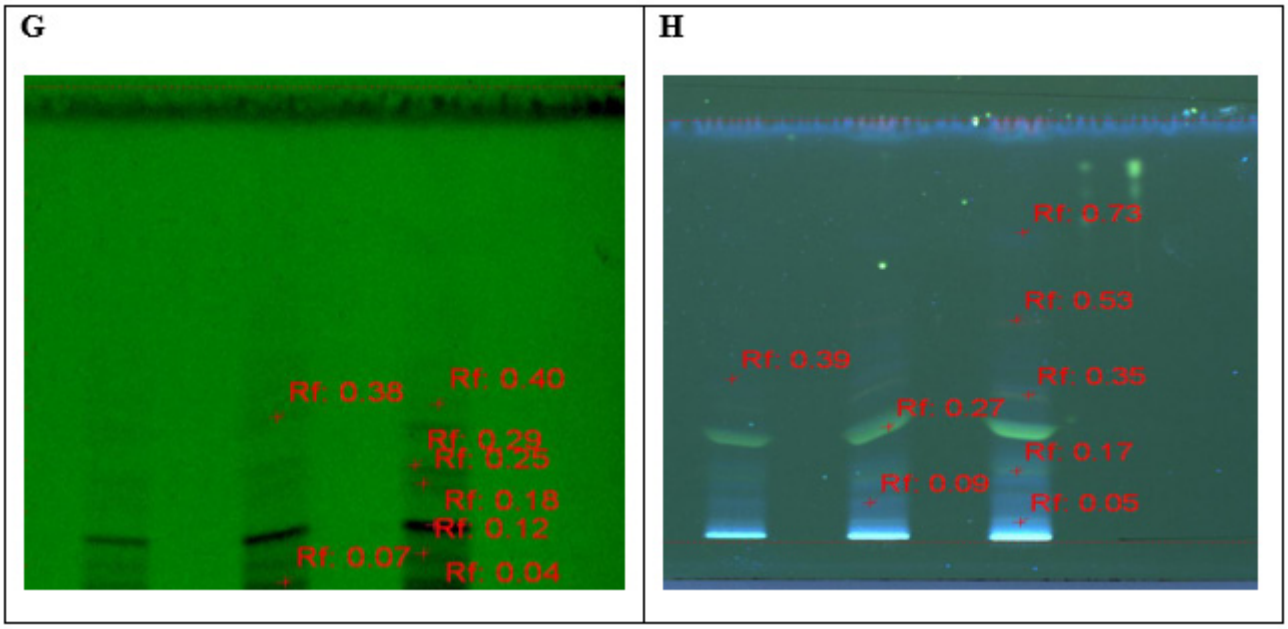

Figure 4: HPTLC Chromatograms of decoction prepared using 500g of *Dry* sample of *Tinospora cordifolia* Linn. stems {@ 254 (G) and 366 nm (H)}.

The qualitative chemical analysis of hexane, chloroform, ethyl acetate and methanol extracts of fresh and dry stem samples of *Tinospora cordifolia* Linn. showed almost similar results. Hence, conclusive evidence was not found in the preliminary phytochemical analysis.

The HPTLC results showed more spots in the decoction prepared (methanolic extract) with the fresh sample in the required quantity than in double quantity. i.e., 8 spots each were obtained at 254 nm and 366 nm in the fresh sample, but the spots obtained for decoction prepared with 500 g (double quantity) of fresh Guduchi sample were 8 and 7 at 254 nm and 366 nm. These results support the classical reference that doubling the quantity is not required for the Guduchi if taken in fresh form compared to dry form. Further, more HPTLC spots were obtained in extracts (hexane, ethyl acetate, chloroform and methanol) of fresh samples of Guduchi when compared to dry samples. This evidence seconds the Ayurvedic principle of drug collection. i.e., this drug should be mandatorily taken in fresh form whenever used.

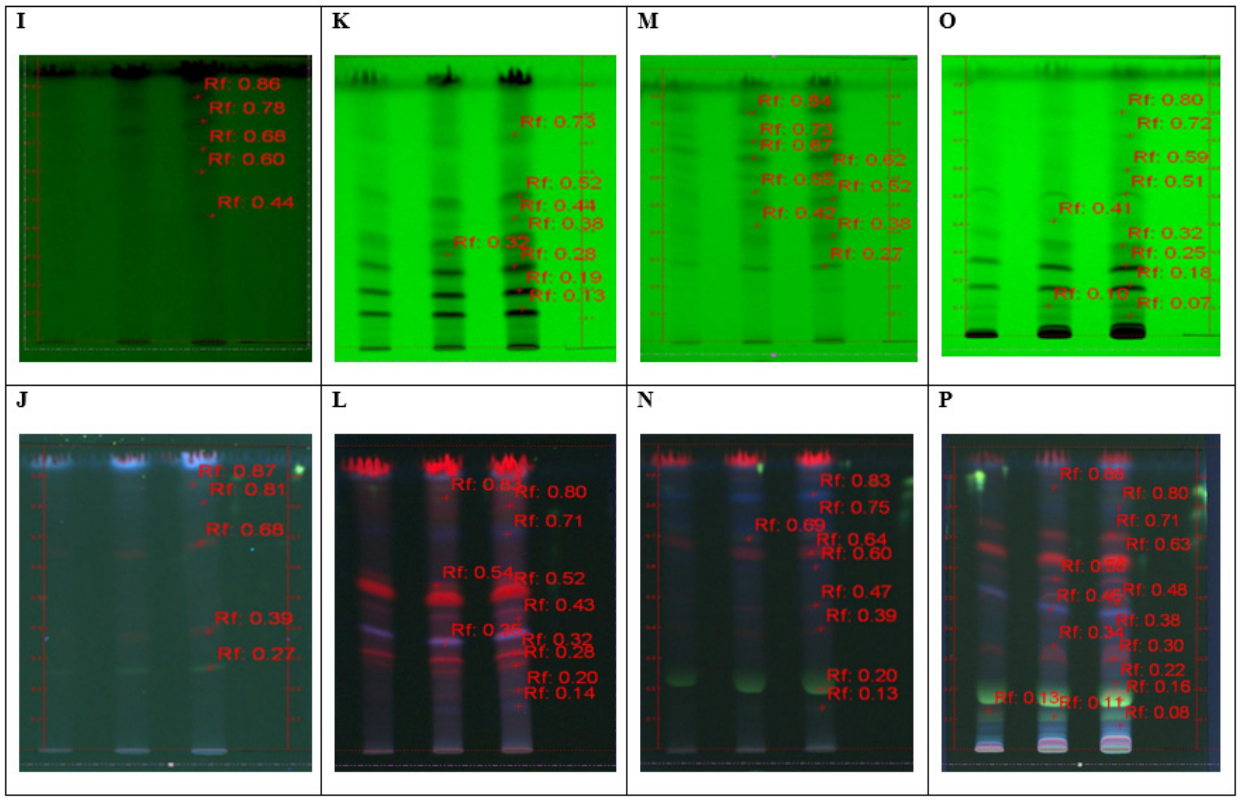

Figure 5: HPTLC Chromatograms of hexane {@ 254 (I) and 366 nm (J)}, ethyl acetate {@ 254 (K) and 366 nm (L)}, chloroform {@ 254 (M) and 366 nm (N)} and methanol {@ 254 (O) and 366 nm (P)} extract of *Fresh* sample of *Tinospora cordifolia* Linn. stems.

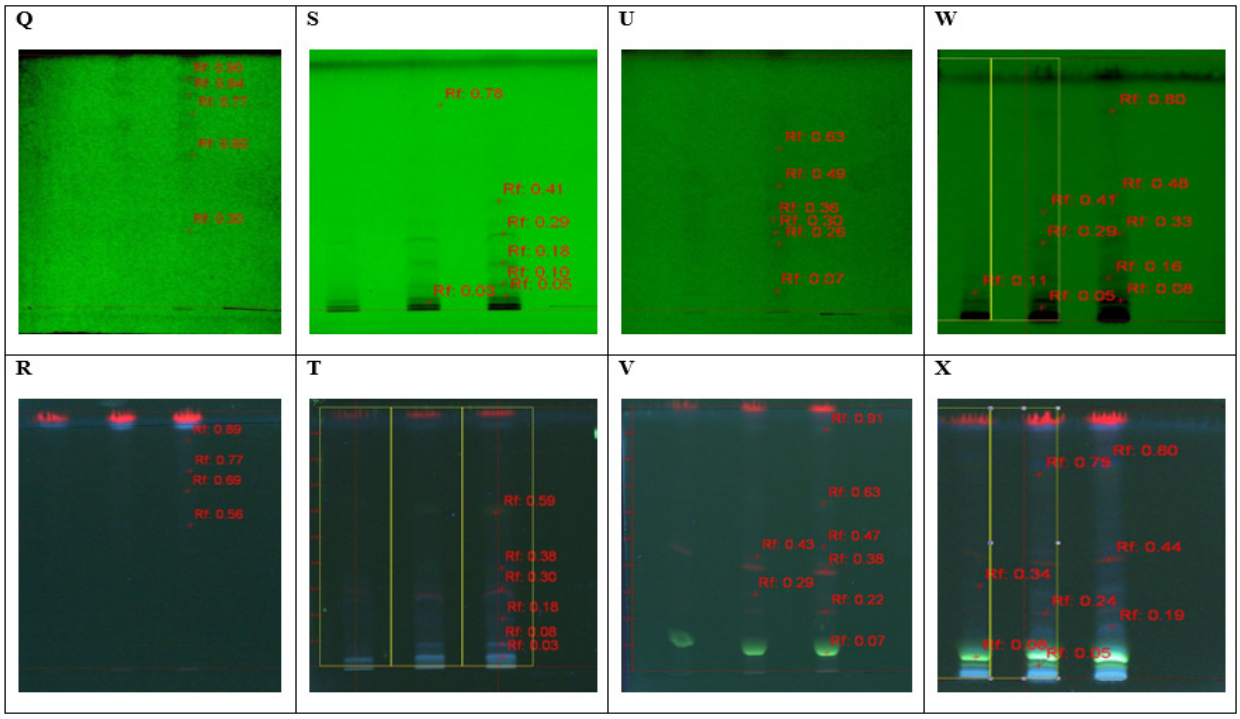

Figure 6: HPTLC Chromatograms of hexane {@ 254 (Q) and 366 nm (R)}, ethyl acetate {@ 254 (S) and 366 nm (T)}, chloroform {@ 254 (U) and 366 nm (V)} and methanol {@ 254 (W) and 366 nm (X)} extract of *Dry* sample of *Tinospora cordifolia* Linn. stems.

Table 4: A Compiled Number of R_f values obtained from the different Dry and Fresh Guduchi stem samples extracts and the decoction prepared with the required quantity (250 g) and double quantity (500 g).

Sl. No.	Name of the Sample/Type of extract	Fresh Sample		Dry Sample	
		@256 nm*	@ 366 nm	@ 256 nm	@ 366 nm
1	Methanolic extract of decoction prepared with 250 g** Guduchi stems.	8	8	4	3
2	Methanolic extract of decoction prepared with 500 g Guduchi stems.	8	8	8	8
3	Hexane extract.	5	5	5	4
4	Ethyl acetate extract.	8	11	7	6
5	Chloroform extract.	9	9	6	8
6	Methanolic extract.	10	15	9	8

*Nano metre, ** gram.

In addition, the authors made a new observation while analyzing the HPTLC spots obtained from the decoction prepared with dry samples. i.e., the decoction (methanolic extract) prepared with double the quantity (500 g) of Guduchi showed more spots than that prepared with the required quantity (250 g). Due to practical feasibility, the manufacturers usually take the Guduchi samples in dry form to prepare formulations. However, as per the results of the HPTLC analysis, the phytoconstituents in the dry samples are very low; but still, the double-quantity usage may provide more phytoconstituents in decoction. Hence, if Guduchi is taken in decoction in dry form, it can preferably be taken in double quantity. Still, this study has limitations because only preliminary phytochemistry and HPTLC analysis have been evaluated. So, more elaborative studies and quantitative analysis are needed to get conclusive evidence. Also, as per the published article on the validation of wet and dry drug collection principles in the preparation of shatavari ghrita through quantitative estimation of shatavarin IV, the results coincided with the classical references. Shatavari is also an exceptional drug like Guduchi, which all the guidelines mentioned earlier. Hence, as far as possible, these exceptionals are to be taken in fresh form in formulations. If it is intended to be used in dry form, it is better to be used after getting valuable supporting scientific evidence.

CONCLUSION

This study enhances our understanding of Ayurvedic drug usage, suggesting usage of fresh Guduchi samples in decoctions wherever feasible. For dry samples, doubling the quantity is suggested for optimal efficacy. This study also provides a foundation for further exploration of its medicinal efficacy.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of Interest.

ABBREVIATIONS

mL: Millilitre; **HPTLC:** High Performance Thin Layer Chromatography; **g:** Gram; **R_f:** Retardation factor; **nm:** Nanometre; **TLC:** Thin Layer Chromatography, **µ:** Micron; **PTFE:** Polytetraflouroethylene; **µL:** Micro Litre; **mm:** Millimetre; **°C:** Degree Celsius.

AUTHOR CONTRIBUTIONS

Sree Deepthi G N[1]- Conceptualization, Literature search, data analysis, drafting, review and editing. **Thirupataiah Boini**[2]- Data collection, data analysis, drafting, review and editing. **Praveen Balakrishnan**[1]- Review and editing. **Krishna Kumar V**[3]- Review and editing. **Sinimol T P**[1]- Literature search, review and editing.

SUMMARY

The Ayurvedic herb *Tinospora cordifolia* Linn., also known as Guduchi, is an exceptional drug mandatorily used in fresh form in formulations as per classical references. Generally, when fresh drugs are used in formulations, they should be taken in double quantity. But for exceptional drugs like Guduchi, a double quantity is not required. In the modern era, according to its feasibility, manufacturers usually use it in dry form to prepare formulations. In this study, the preliminary phytochemistry and HPTLC analysis of fresh and dry samples of Guduchi has been analyzed to find out the logic behind the usage in fresh form. The results suggest that it is better to use these drugs in fresh form in formulations which corresponds to the guidelines given in classical Ayurvedic textbooks.

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