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PHYTOCHEMICAL EVALUATION OF LYOPHILIZED JUICE OF *DURVA* (*CYNODON DACTYLON* (L.) PERS.) AS A STABLE SUBSTITUTE FOR *SWARASA*: A COMPARATIVE STUDY WITH *CHURNA*

[1]Aswathy P Kumar , [2] Resmi R
[1]PG Scholar, Dept of Dravyaguna Vigyan, Mannam Ayurveda Co-operative Medical College Kerala University of Health Sciences, Kerala.
[2]Associate Professor, Dept of Dravyaguna Vigyan, Mannam Ayurveda Co-operative Medical College, Kerala University of Health Sciences, Kerala.
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ABSTRACT

Aim: *Durva* (*Cynodon dactylon* (L.) Pers.) is a creeping grass considered auspicious in rituals, is listed among the *Varnyadashemani* in the *Charaka Samhita* and also included in *Dasapushpam*. The study aims to evaluate the impact of lyophilization on the phytochemical composition and chemical complexity of *Durva (Cynodon dactylon* (L.) Pers.*)* by comparing lyophilized form with *churna* (whole-plant powder). **Materials & Methods:** Fresh *Durva* (*Cynodon dactylon* (L.) Pers.) plants were collected, thoroughly washed, and used to prepare *swarasa*, which was then subjected to lyophilization**.** Whole-plant powder was prepared by shade-drying and pulverization. Physicochemical parameters, extractive values, and qualitative phytochemical screening of lyophilized form of *Durva (Cynodon dactylon* (L.) Pers.) and *churna* (whole-plant powder) were conducted. Chromatographic fingerprinting was performed using Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC).**Results:** Lyophilized form demonstrated higher total ash and loss on drying, higher alcohol- and water-soluble extractive values, and enhanced detectability of phytochemicals compared to whole-plant powder. TLC and HPTLC profiling revealed sharper, more intense bands and a broader metabolite range in the lyophilized form, indicating superior retention of bioactive compounds.
Conclusion: Lyophilization effectively preserves the phytochemical integrity and enhances the chemical complexity of *Durva (Cynodon dactylon* (L.) Pers., producing a stable, potent, and standardized extract suitable for pharmacological studies and therapeutic applications.
Key-words: *Cynodon dactylon*, *Durva*, lyophilization, *swarasa,* fresh juice, substitute

INTRODUCTION: *Durva* (*Cynodon dactylon* (L.) Pers.) is a hardy, perennial, creeping grass that grows widely across the country, reaching altitudes of up to 2440 meters extensively used in traditional medicine systems, particularly *Ayurveda*.[1]It posses *Madhura-Tikta-Katu Rasa* (sweet, bitter, and pungent tastes); *Laghu Guna* (light quality); *Shita Veerya* (cooling potency); *Madhura Vipaka* (sweet post-digestive effect); and is *Kapha-Pittahara* (pacifies *Kapha* and *Pitta doshas*)." The recommended dose of *swarasa*(fresh juice) is 10–20 ml.[1]The healing properties of *Durva* (*Cynodon dactylon* (L.) Pers) are attributed to its rich array of natural phytochemicals.

However, the method of processing can significantly influence the quality and efficacy of these bioactive compounds. Conventional methods such as oven drying at high temperatures, sun drying, or shade

drying can alter phytochemical composition and reduce therapeutic potential, while gentler methods like low-temperature oven drying or shade drying help retain their integrity.[2]

Swarasa (fresh juice) although potent, is highly perishable, limiting its shelf life and practical use in standardized formulations. Since *swarasa* (fresh juice) is the commonly used dosage form of *Durva (Cynodon dactylon* (L.) Pers.*)* in *Ayurveda,* lyophilized form has been adopted as a modern alternative to retain its phytochemical integrity and enhance stability, while preserving the therapeutic potential of the *swarasa* (fresh juice)**.** Among modern preservation techniques, lyophilization stands out by removing water without heat, thereby maintaining phytochemical composition and therapeutic potential, producing a stable and potent form suitable for medicinal applications.[3]

Lyophilization is a widely adopted strategy in the pharmaceutical industry to enhance the stability and long-term storage of labile drugs, particularly biopharmaceuticals.[4] It is a low-temperature vacuum dehydration method that removes water while minimizing thermal and oxidative degradation. It preserves thermo labile phytochemicals, ensuring a potent, stable, and standardized herbal product for therapeutic use.

However, systematic comparative studies evaluating the impact of lyophilization on the phytochemical composition of *Durva* (*Cynodon dactylon* (L.) Pers) are limited. The present study aimed to assess the effect of lyophilization on the phytochemical profile of *Durva* (*Cynodon dactylon* (L.) Pers) by comparing lyophilized form with *churna* (whole plant powder). Physicochemical parameters, qualitative phytochemical screening, and chromatographic fingerprinting using Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) were performed.

AIM AND OBJECTIVES OF THE STUDY

Aim

To evaluate the effect of lyophilization on the phytochemical composition of *Durva* (*Cynodon dactylon* (L.) Pers.) by comparing the phytochemical parameters and chemical complexity of its lyophilized preparation with the traditional *churna* (whole-plant powder).

Objectives

- To prepare the lyophilized form and *churna* of Durva (Cynodon dactylon (L.) Pers.).
- To perform phytochemical screening of both preparations.
- To compare the phytochemical parameters of the two preparations to evaluate the effect of lyophilization.

MATERIALS AND METHODS

The whole plant of *Durva* (*Cynodon dactylon* (L.) Pers.) was collected from Pandalam, Kerala and authenticated by the HOD, Dept of Botany, NSS College, Pandalam on October 2024 and macro–microscopic examination was performed at Dept of Dravyaguna Vigyan,Mannam Ayurveda Co-operative Medical College, Pandalam.

Freshly collected plants were washed thoroughly, before preparing the lyophilized form and powder. For extract preparation, 1kilogram fresh plant material was blended with distilled water (1200 millilitre) to obtain *swarasa*.[5]

The obtained *swarasa* was transferred into sterile lyophilization vials and pre-frozen at −20°C to −80°C for 12–24 hours. The frozen samples were lyophilized using a freeze dryer with primary drying at −30°C to −40°C under 100–200 m Torr vacuum, followed by secondary drying at +20°C below 100 m Torr for 24–48 hours., yielded 22 grams of lyophilized extract which was approximately 2.2%.[6] Whole-plant powder was prepared by shade-drying 01 (one)kilogram of fresh plant material yielding approximately 750 grams

followed by pulverization into a fine powder.

Physicochemical parameters, including foreign matter, total ash, acid-insoluble ash, water-soluble ash, loss on drying at 105 °C, and pH of aqueous solution (1%), were determined following the Ayurvedic Pharmacopoeia of India guidelines.[7]

Hot and cold extractive values were determined using the solvents alcohol and water and the qualitative analysis of the extractives were performed to detect major phytoconstituents. TLC and HPTLC analysis were conducted on silica gel 60 F_{254} plates using toluene: ethyl acetate: formic acid (2:5:0.1), with chromatograms visualized under UV, derivatized, scanned densitometrically, and Rf values recorded for fingerprinting.

Observations and Results

Preliminary Phytochemical Evaluation

The preliminary phytochemical evaluation of *Durva* (*Cynodon dactylon* (L.) Pers.) was conducted to compare the characteristics of the *churna* with its lyophilized form. The study encompassed physicochemical profiling, extractive value estimation, qualitative phytochemical screening, and chromatographic fingerprinting (TLC and HPTLC).

- **Physicochemical Evaluation**

This table summarizes the physicochemical characteristics of *Durva* (*Cynodon dactylon* (L.) Pers.), comparing the whole plant powder and its lyophilized form, including ash values, moisture content, and pH.

Table 01: Physicochemical parameters of *churna* and lyophilized form of *Durva* (*Cynodon dactylon* (L.) Pers).

Parameter	***Churna***	**Lyophilized Form**
Foreign matter	Nil	Nil
Total ash	4.5%	13.7%
Acid insoluble ash	2.5%	Nil
Water soluble ash	8%	8.72%
Loss on drying (105°C)	7 % w/w	18.64 % w/w
Qualitative pH	Slightly acidic	Neutral
Quantitative pH	6	7

- **Alcohol and water-soluble extractives of *churna* and lyophilized form of *Durva (Cynodon dactylon* (L.) Pers.)**

Extractive values were determined using the solvents alcohol and water under both hot and cold conditions for the *Churna,* whereas cold alcohol and water extracts were analyzed for the lyophilized form.

Table 02: Extractive values of *churna* and lyophilized form of *Durva (Cynodon dactylon* (L.) Pers.)

Extract	***Churna***	**Lyophilized Form**
Cold alcohol soluble	3%	15.90%
Hot alcohol soluble	3.2%	-
Cold water soluble	12%	24.41%
Hot water soluble	15%	-

The lyophilized form showed a significantly higher extractive percentage in both solvents, indicating improved solubility and enhanced retention of phytoconstituents.

Qualitative analysis of the extractives of *churna* and lyophilized form of *Durva (Cynodon dactylon* (L.) Pers.)

Different solvent extracts were subjected to standard chemical tests for major phytochemical groups including alkaloids, phenolics, flavonoids, saponins, steroids, carbohydrates, and proteins.

Table 03: Qualitative analysis of *churna* and lyophilized form of *Durva (Cynodon dactylon* (L.) Pers.)

Name of Test	*Churna* of *Durva (Cynodon dactylon* (L.) Pers.)				Lyophilized Form of *Durva (Cynodon dactylon* (L.) Pers.*)*	
	Alcohol (Hot extract)	Alcohol (Cold extract)	Water (Hot extract)	Water (Cold extract)	Alcohol (Cold extract)	Water (Cold extract)
Alkaloids						
Mayers	+	+	-	-	+	-
Dragendorff's	+	+	-	-	+	-
Steroids	+	+	-	-	+	-
Phenolic compounds						
Ferricchloride	+	+	-	-	+	-
Lead acetate	+	+	-	-	+	-
Flavonoids	+	+	-	-	+	-
Carbohydrates						
Benedict's	+	+	+	+	+	-
Fehling's	+	+	+	+	+	-
Saponins	-	-	-	-	-	+
Proteins	-	+	+	-	-	+

((+) indicates present and (–) indicates absent)

The whole plant powder demonstrated moderate presence of alkaloids, phenolics, flavonoids, steroids, and carbohydrates, whereas the lyophilized form displayed a broader and more consistent phytochemical presence particularly in alcohol extracts. Water extracts of the lyophilized form showed additional detection of saponins and proteins, indicating that lyophilization enhances the stability and detection of water-soluble phytochemicals.

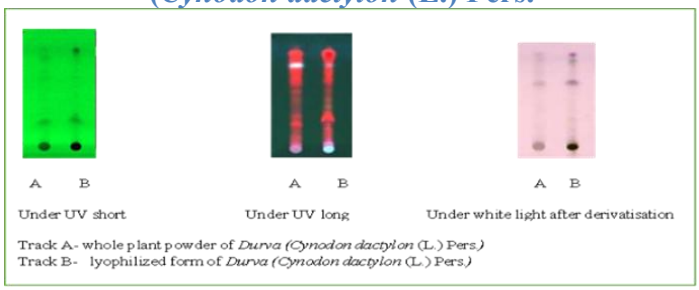
- **TLC profile of alcoholic extract of *churna* and lyophilized form of *Durva* (*Cynodon dactylon* (L.) Pers).**

TLC profiling of alcoholic extracts revealed distinct bands under UV short wavelength (254 nm), UV long wavelength (366 nm), and visible light after derivatization(575nm).

Table 04: TLC Profiles of alcoholic extract of *churna* and lyophilized form of *Durva* (*Cynodon dactylon* (L.) Pers).

Solvent System	Extract	UV Detection	Rf Value
Toluene:Ethyl acetate: Formic acid	Alcoholic extract of whole plant powder of *Durva* (*Cynodon dactylon* (L.) Pers.)	254 nm	0.22, 0.35
		366 nm	0.24, 0.43, 0.73
		575 nm	0.40, 0.52, 0.66
Toluene:Ethyl acetate: Formic acid	Alcoholic extract of lyophilized form of *Durva* (*Cynodon dactylon* (L.) Pers.)	254 nm	0.21, 0.40
		366 nm	0.42,0.55, 0.61,0.75
		575 nm	0.54, 0.65

Figure 01: TLC profile of alcoholic extract of *churna* and lyophilized form of *Durva* (*Cynodon dactylon* (L.) Pers.



The whole plant powder showed fewer but clear spots, whereas the lyophilized extract displayed more visible and fluorescent bands, indicating a slightly richer presence of phytochemicals. Minor differences in Rf values were observed between the two samples, reflecting small variations in polarity.

High-Performance Thin Layer Chromatography (HPTLC)

The HPTLC analysis provided a detailed fingerprint of both extracts. The lyophilized sample consistently yielded more peaks with larger peak areas under all detection wavelengths (254 nm, 366 nm, and 575 nm).

Table 05: HPTLC Fingerprint profile of alcoholic extract of *Churna* and lyophilized form of *Durva* (*Cynodon dactylon* (L.) Pers).

Wavelength	***Churna***	**Lyophilized Form**
254 nm	4 peaks, area 7,330.9 AU; major Rf 0.47	11 peaks, area 10,018.4 AU; major Rf 0.47
366 nm	5 peaks, area 24,808.5 AU; major Rf 0.98	7 peaks, area 62,834.4 AU; major Rf 0.83
575 nm	6 peaks, area 7,168.7 AU; major Rf 0.49	9 peaks, area 15,276 AU; major Rf 0.96

Figure 02: HPTLC Fingerprint profile of alcoholic extract of *Churna* and lyophilized form of *Durva* (*Cynodon dactylon* (L.) Pers.

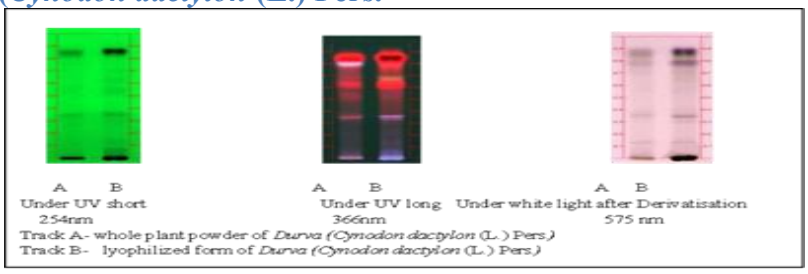
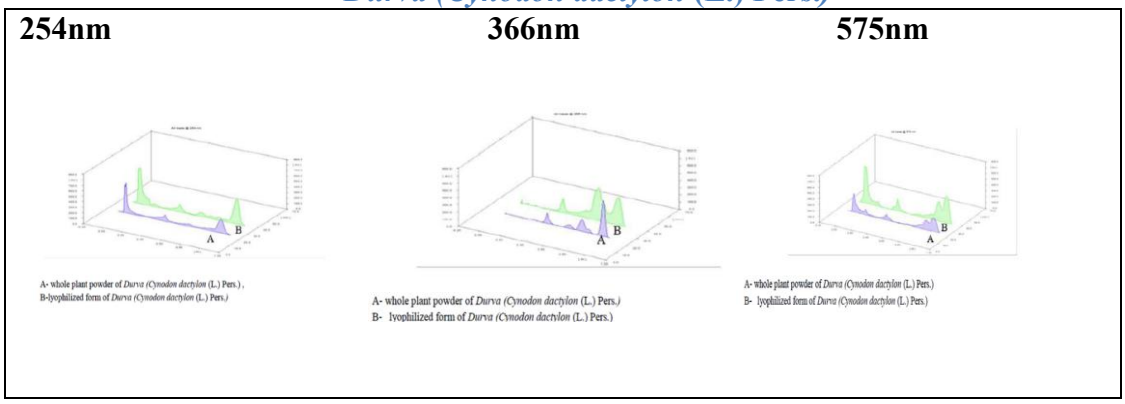


Fig 03. 3D overlay Chromatogram of alcoholic extract of *churna* and lyophilized form of *Durva (Cynodon dactylon* (L.) Pers.*)*



Lyophilized form produced sharper and more intense chromatographic responses, indicating better retention of bioactive constituents such as flavonoids, phenolics, and terpenoids.

DISCUSSION

The comparative evaluation of the *churna* and the lyophilized form of *Durva (Cynodon dactylon (L.) Pers.)* demonstrated significant differences in physicochemical properties, extractive yields, and phytochemical profiles.

Physicochemical analysis revealed that both samples were free from foreign matter, the lyophilized sample exhibited a higher total ash content and moisture percentage, suggesting greater retention of inorganic salts and hygroscopic components. The lyophilized preparation possessed a notably higher total ash value (13.7%) compared to the raw whole plant powder (4.5%).

This increase suggests enhanced retention of inorganic constituents following freeze-drying. The complete absence of acid-insoluble ash in the lyophilized form further indicates greater purity, with minimal siliceous or earthy contaminants. Comparable water-soluble ash values in both forms confirm preservation of essential electrolytes and mineral salts irrespective of processing. The higher loss on drying observed in the lyophilized sample reflects its porous structure, characteristic of freeze-dried materials, which enhances moisture absorption.

A marked enhancement in extractive values was observed in the lyophilized sample, with alcoholic and aqueous extractives rising to 15.90% and 24.41%, respectively, compared to 3–3.2% and 12–15% in the *churna*. This indicates that lyophilization concentrates phytoconstituents and preserves heat-sensitive compounds, improving solvent accessibility and extraction efficiency. The predominance of water-soluble extractives in both forms reflects the abundance of hydrophilic phytochemicals such as flavonoids, phenolics, glycosides, and carbohydrates in *Durva (Cynodon dactylon (L.) Pers.)*.

Chromatographic profiling further demonstrated the superior phytochemical richness of the lyophilized form. TLC analysis showed a greater number of fluorescent and derivatized bands in the lyophilized form, suggesting improved detectability and stability of several phytochemicals. Minor shifts in Rf values between the two forms indicate subtle variations in polarity resulting from processing differences.

HPTLC profiling offered a more detailed comparative fingerprint, showing that the lyophilized extract exhibited a higher number of peaks and larger peak areas at all detection wavelengths (254 nm, 366 nm, and 575 nm). Sharper and more intense

peaks in the lyophilized form indicate better separation and preservation of active secondary metabolites. Major peaks in the higher Rf ranges (~0.65–1.00) confirmed the presence of important phytoconstituent groups such as flavonoids, phenolics, and terpenoids. The enhanced chemical complexity of the lyophilized form correlates with its higher extractive values and supports its stronger potential for biological activity.

Overall, the combined findings indicate that lyophilization significantly improves the phytochemical yield, purity, and chromatographic clarity of *Durva (Cynodon dactylon (L.) Pers.)*. This processing method preserves a broader range of active constituents and enhances extraction efficiency. The improved phytochemical richness and purity support its therapeutic usefulness and indicate that the lyophilized form may be used as a reliable substitute for *Swarasa*.

Lyophilization offers major advantages in phytochemical research and herbal product development. By removing water under low temperature and vacuum, it reduces thermal degradation, oxidation, and enzymatic hydrolysis. The technique preserves bioactive compounds, improves extract solubility and yields. It also enables long-term storage with minimal loss of constituents, ensuring consistent therapeutic efficacy.[8]

CONCLUSION

The study demonstrates that lyophilization significantly enhances the phytochemical richness, extractive efficiency, and chromatographic profile of *Durva* (*Cynodon dactylon* (L.) Pers.), compared to its powder form. These findings underscore the value of lyophilization as a preservation and extraction strategy, ensuring high-quality plant extracts suitable for pharmacological studies, quality control, and potential therapeutic applications.

Declaration

The authors hereby declare that no artificial intelligence (AI) technologies or AI-assisted tools were utilized in the preparation of this manuscript.

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Corresponding Author: Dr. Resmi.R Associate Professor, Dept of Dravyaguna Vigyan, Mannam Ayurveda Co-operative Medical College, Kerala University of Health Sciences, Kerala.
Email: drresmir@gmail.com

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