Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
Impact Factor (RJIF): 5.94
www.plantsjournal.com
JMPS 2025; 13(6): 45-51
© 2025 JMPS
Received: 20-08-2025
Accepted: 25-09-2025

Dr. G Veda Priya
Professor, Aditya College of Pharmacy, Surampalem, Andhra Pradesh, India

Dr. K Swathi Priya
Associate Professor, Srinivasarao College of Pharmacy, Visakhapatnam, Andhra Pradesh, India

S Amala
Associate Professor, Aditya College of Pharmacy, Surampalem, Andhra Pradesh, India

Corresponding Author:
Dr. G Veda Priya
Professor, Aditya College of Pharmacy, Surampalem, Andhra Pradesh, India

Phytochemical investigation and HPTLC of *Cycas revoluta*-based topical ointment

G Veda Priya, K Swathi Priya and S Amala

DOI: https://www.doi.org/10.22271/plants.2025.v13.i6a.1975

Abstract
Cycas revoluta, commonly known as the Sago palm, is an ornamental plant native to Southeast Asia and is classified as a gymnosperm under the Cycadaceae family. Historically, it has been utilized by various tribal communities for diverse medicinal and culinary applications. Distinct parts of the Sago palm are employed in treating numerous ailments, including estrogen-dependent carcinoma, different forms of cancer, liver tumors, diarrhea, dysentery, flatulence, vomiting, hemorrhoids, and wounds. The current research focuses on preliminary phytochemical screening, fluorescence analysis, and the interaction of chemical reagents with various phytoconstituents found in different extracts of the plant. Additionally, High-Performance Thin-Layer Chromatography (HPTLC) studies have been conducted using ethanolic extracts from the leaves to further explore its components. The preparation of the ointment base involved using a levigation method to integrate the herbal extract into the base, resulting in the formulation of a herbal ointment. Upon formulation, various physicochemical parameters were assessed to ensure its quality, including color, odor, pH level, spreadability, extrudability, consistency, solubility, and washability, providing an understanding of the ointment's characteristics and performance.

Keywords: *Cycas revoluta*, phytochemical screening, HPTLC studies, herbal ointment

1. Introduction
Over the past thirty years, the use of herbal medicinal products and supplements has seen a significant rise, with at least 80% of the global population depending on them for various aspects of primary healthcare [1]. The field of traditional medicine is particularly esteemed, as it involves medicinal plants and the beneficial chemical compounds they contain, offering therapeutic potential for treating a range of health conditions. These natural remedies play crucial roles in preventing, diagnosing, improving, or treating both physical and mental illnesses [2]. This trend has led to a growing demand for plant-derived products, especially in developed countries [3]. These products are gaining popularity for their use in medicine, nutraceuticals, and cosmetics. The commercialization of certain nutraceuticals emphasizes their therapeutic effects on various physiological disorders. Currently, over 100 drugs derived from natural products are undergoing clinical trials. Remarkably, among the 252 drugs listed as essential by the World Health Organization (WHO), 11% are solely plant-based [4]. While isolating active ingredients from each herb is possible, it is an extremely labor-intensive and costly process, making it impractical for manufacturers [5]. In the current study, the focus was placed on the leaves of *Cycas revoluta*, a plant known for its rich medicinal properties. The healing potentials of these leaves include antiviral [6], astringent diuretic [7], antioxidant [8], antidiabetic [9], antimicrobial [10], antibacterial [11], antifungal [12], cytotoxic [13], anticancer [14], and antirheumatic effects [15]. Previous phytochemical research into the plant's leaflets has successfully isolated various beneficial compounds. These include flavonoids, such as naringenin [16] and pruning [18] biflavonoids, like amentoflavone [18] and other phenolic and alcoholic compounds, including lariciresinol, protocatechuic acid, and vomifoliol. These findings underscore the promising therapeutic value inherent in *Cycas revoluta*.

Collection of leaves
The leaves from the *Cycas revoluta* (Sago palm) were collected were gathered from the local gardens at Aditya Educational Institutions in Surampalem in March 2025. These leaves were then carefully separated and washed with distilled water to ensure cleanliness. Following this, they were dried in the shade to preserve their quality before being pulverized for further use.

Extraction of Leaves: The powder of the *Cycas revoluta* thunb were soaked separately in different solvents. The powdered material was stirred using a sterile glass rod for every 24hrs. The coarse powder was allowed to macerate with ethanol at room temperature for five days. The resulting extract was then concentrated using a rotary evaporator under vacuum at a controlled temperature of 45°C, ensuring the material was completely dried and stored in a desiccator.

Table 1: Details of Extraction

Plant Material	Solvent used	Volume of the solvent	Weight of the extract
Leaves (*Cycas revoluta*) 100 gm	Ethanol (70%)	500ml	14 g
Leaves (*Cycas revoluta*) 100 gm	Ethyl acetate	500ml	11g

Preliminary Phytochemical Screening [19-20]

Table 2: Phytochemical Screening

Phytoconstituents	Name of the Test	Ethanol	Ethyl acetate
Akaloids	Mayer's test	+ ve	- ve
	Wagners test	+ ve	- ve
	Dragendroff's test	+ ve	- ve
	Hagers test	+ ve	- ve
Triterpenoids	Salkowski test	- ve	+ve
Steroids	Lieberman buchard test	- ve	- ve
Tannins and Phenols	Ferric chloride test	- ve	+ve
Flavanoid	Shinoda test	+ ve	- ve
	Zn Hcl reduction test	+ ve	- ve
	Lead acetate test	+ ve	- ve
	Alkaline reagent test	+ ve	- ve
Saponins	Foam test	- ve	- ve
Carbohydrates	Molisch test	- ve	- ve
	Fehlings test	- ve	- ve
	Benedicts test	- ve	- ve
Glycosides	Baljet test	- ve	- ve
	Legal test	- ve	- ve
	Killer kellani test	- ve	- ve
Amino acids	Ninhydrin test	- ve	- ve

Fluorescence analysis

Fluorescent colours are unique to each compound, and plant materials exhibit varying colour changes when exposed to different chemicals. Certain plant constituents demonstrate distinctive fluorescence visible in daylight. Addition of different reagents results in the conversion into fluorescent derivatives or decomposition products. For evaluation crude drugs they are often assessed qualitatively as an important pharmacognostic parameter [21].

Small quantity of dried, finely powdered and dried leaf sample was taken in a small quantity on a clean microscope slide. Subsequently, 1-2 drops of a freshly prepared solution were added, and the mixture was gently tilted to ensure proper mixing. The slide was rested for 1-2 minutes observed was in the UV chamber at wavelengths of 250 nm (short) and 365 nm (long) [22]. The different colours seen with the application of various reagents under these different radiation wavelengths were then carefully recorded Table no: 3.

Table 3: Fluorescence analysis

S.No	Reagents	Visible light	UV (254)	UV (3665nm)
1	Powder as such	Green	Brown	Greenish yellow
2	Powder + 5% iodine	Green	Brown	Blackish brown
3	Powder + Nacl	Green	Brown	Blackish green
4	Conc. Sulphuric acid	Black	Black	Black
5	50% Sulphuric acid	Black	Blackish red	Greenish
6	Conc. HCl	Black	Blackish red	Greenish
7	50% Conc. Hcl	Black	Blackish red	Light brown
8	10% Sodium hydroxide	Greenish brown	Black	Blackish red
9	5% Ferric chloride	Greenish brown	Black	Dark green
10	With water	Dark brown	Light brown	Light brown

Microscopical characters

Preparation of specimens: To prepare the specimens for microscopic examination, freshly collected samples of leaves were initially preserved in a solution consisting of Formalin (5 ml), Acetic acid (5 ml), and 70% ethyl alcohol (90 ml). After a 24-hour period of fixation, the specimens were carefully sliced into transverse sections by hand. These sections were then stained using safranine and phloroglucinol to enhance visibility. Finally, they were mounted in glycerin, allowing them to be observed clearly under a microscope [23].

The anatomical characters are listed are as follows

- **Epidermis:** It is single layered with cuticle. Lignin and waxy substances are absent. Cells are isodiametric.
- **Hypodermis:** It is present below the epidermis. In the upper epidermis hypodermis is four layered sclerenchymatous and above lower epidermis it is three layered.
- **Mesophyll:** Located beneath the hypodermis, this structure is highly developed and consists of two distinct layers: the upper palisade and the lower spongy parenchyma. The palisade layer is characterized by radially elongated cells packed with chloroplasts, which are crucial for photosynthesis. In contrast, the spongy parenchyma is composed of loosely arranged cells with significant intracellular spaces, promoting efficient gas exchange. Notably, the palisade cells are found in both the midrib and hypodermis, whereas the spongy parenchyma resides only above the lower epidermis.
- **Transfusion tissue:** Positioned between the palisade tissue and the spongy parenchyma, this layer is comprised of 3 to 5 cell layers, which are transparent in nature. Extending horizontally from the central midrib to the edges, it encompasses the single vascular bundle located within the midrib area.
- **Vascular bundles:** The vascular bundle is conjoint and collateral, exhibiting an open structure with a prominent triangular area of centripetal xylem and two smaller areas of centrifugal xylem. It comprises xylem, cambium, and phloem, but lacks phloem parenchyma and companion cells. Within the xylem, tracheids and parenchyma are present, while xylem vessels are absent.

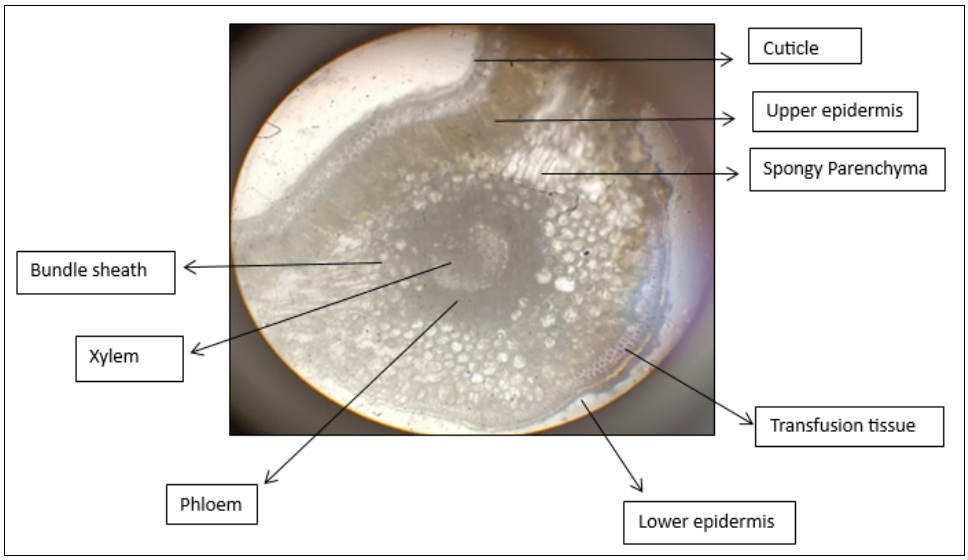

Fig 2: TS of *Cycas revoluta*

Infrared Spectroscopy

Infrared spectroscopy involves studying how infrared light interacts with molecules, which can be explored through measuring absorption, emission, and reflection. When combined with chemometrics, infrared spectroscopy is a valuable method for simple analysis of phytochemicals in medicinal plant extracts. This technique offers several benefits: it is non-destructive, requires only a small amount of sample, provides rapid results, and maintains high accuracy. Infrared technique does not require a reagent, so this method is more eco-friendly. It has been proved to be a powerful analytical tool used in many fields. It is valuable tool for the characterization of plant constituents. It can be near IR region (1250-4000cm-1), mid IR (4000-400cm-1), and far IR (400-20cm-1). The alkaloid components can be identified by the application of IR spectroscopy [24-25].

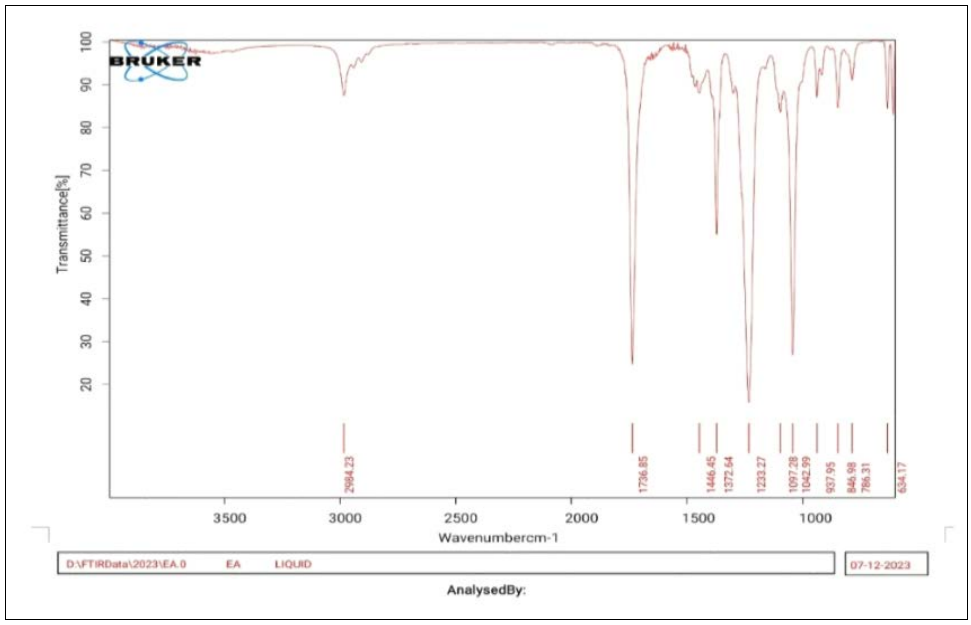

Fig 3: Results of IR spectroscopy of Ethyl acetate

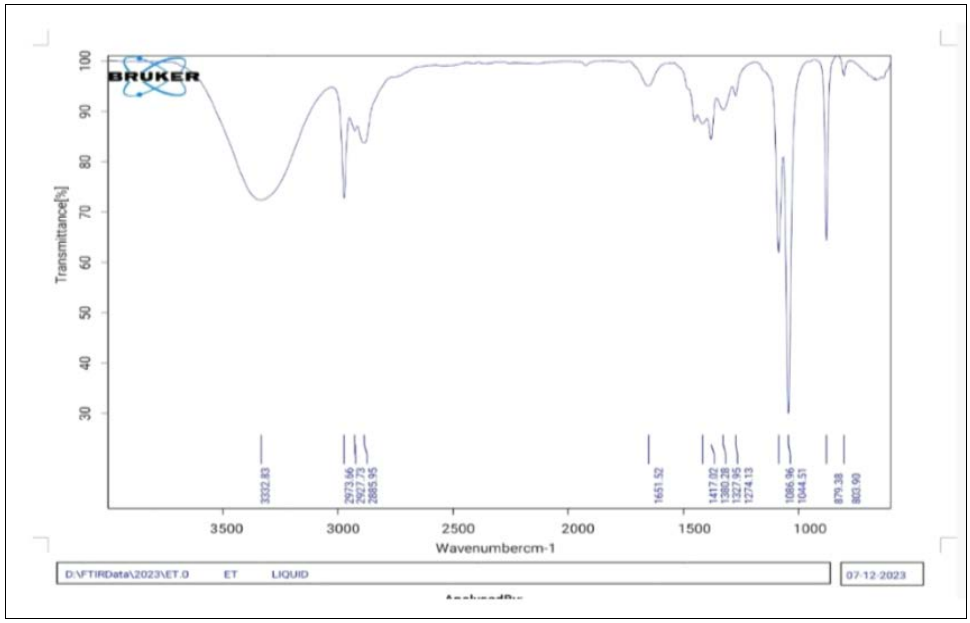

Fig 4: Results of IR spectroscopy of Ethanol

HPTLC studies of *Cycas revolute:* The ethanolic extract of *Cycas revoluta* yielded optimal results in a Toluene, ethyl acetate, and formic acid mixture with a ratio of 5:4:1 by volume in HPTLC studies. This combination was effective in identifying flavonoids using the CAMAG High-Performance Thin-Layer Chromatography (HPTLC) method. The fingerprint method was developed for *Cycas revoluta* extract and the plate was derivatized using flavonoid specific derivatizing reagent. The Presence of Flavonoid was confirmed by observing fluorescent bands after derivatization with Natural Product a reagent (NPA) under 366 nm. The HPTLC images presented in Figure 5 clearly demonstrate that all the components of the samples were distinctly separated, with no evidence of trailing or blurring.

Sample Preparation

***Cycas revoluta* extract (10 mg/ml in Methanol) -** 50 mg of the extract was dissolved in 5 ml of methanol and sonicated for 15 minutes and then centrifuged at 3000 rpm for 10 minutes. The supernatant is collected and used.

- **Diluents Used:** Methanol

Chromatographic conditions

- Stationary Phase Used: TLC Silica Gel 60 F 254 (1.05554.0007)
- Pre-conditioning (if any): N/A
- Development Distance: 70 mm
- Mobile Phase Used: Toluene, ethyl acetate, formic acid 5: 4: 1 (V/V/V)
- Saturation Time: 20 minutes
- Activation time (if any): N/A
- Humidity maintained if any: N/A
- Derivatizing reagent used: Natural Product A Reagent
- Preparation of Derivatizing reagent - Dissolve 1g of 2-aminoetheyl diphenylborinate in 200ml of ethyl acetate.

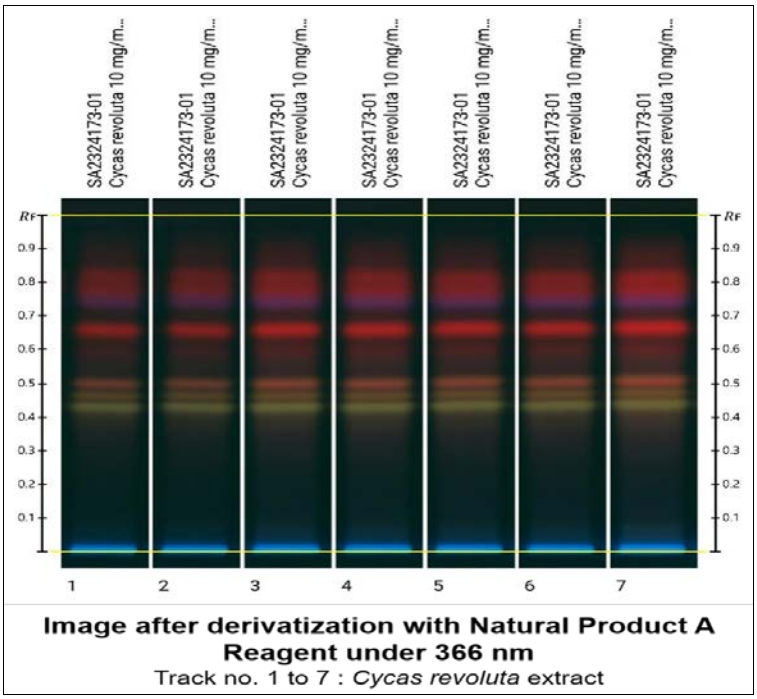

Fig 5: Image under 366 nm with Rf

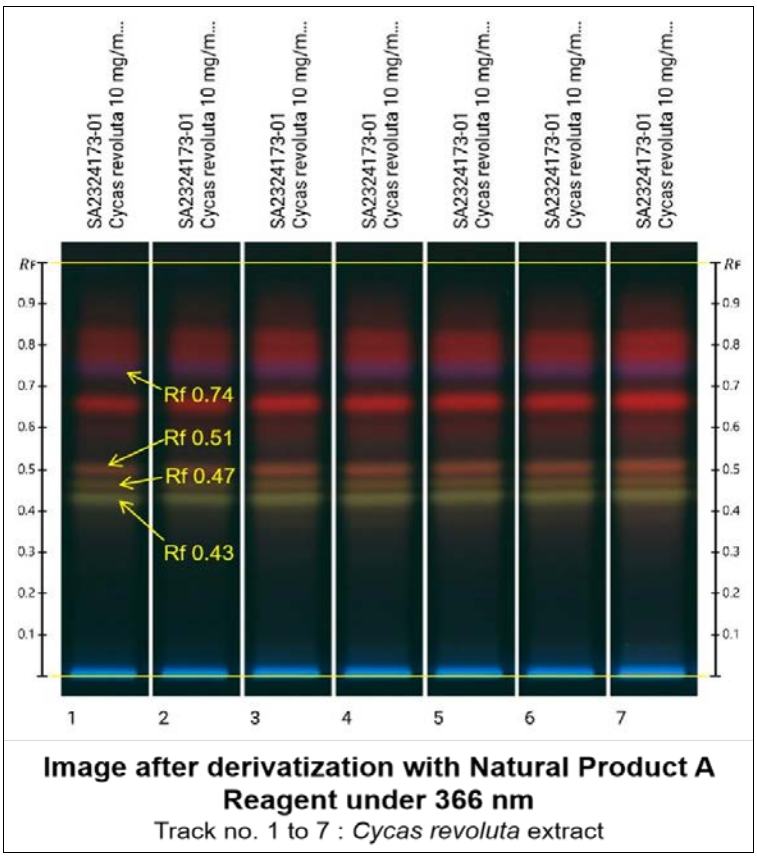

Fig 6: Image under 366 nm with Rf

Observation

- Flavonoid study was carried out for *Cycas revoluta* extract.
- After derivatization with Natural product A Reagent under 366 nm, four fluorescent flavonoid bands were observed as follows:

Conclusion: Detection of flavonoids was successfully done in the given *Cycas revoluta* extract by using CAMAG HPTLC method.

Table 4: R_f value after derivatization at 366nm

Image after derivatization with Natural Product A Reagent under 366 nm			Nature of Compounds
Sample Name	R_f value	Sample Name	From the Rf values and visualization of spots the compounds may be flavanoids or phenolic compounds
Cycas revoluta	0.43	Yellow	
	0.47	Light Brown	
	0.51	Orange	
	0.74	Purple	

Formulation of ointments

a) Hard paraffin was weighed in an evaporating dish over a water bath temperature of 700^0C for preparing the ointment base. The hard paraffin is melted and the remaining ingredients were added and stirred carefully to facilitate uniform mixing and melting followed by cooling of the ointment base.

b) The weighed ethanolic extract of *Cycas revoluta* was prepared by mixing to the ointment base by levigating method, which is the process of integrating the active ingredients with a base by trituration using mortar and pestle. To make a paste with two or three times its weight base is added further until homogeneous ointment is formed. The ointment was subsequently filled into tubes and stored at ambient temperature [26].

Ingredients

Table 5: Formulation of Ointment base

S. No.	Ingredient Name	Quantity
1.	Wool fat	0.5g
2.	Ceto stearyl alcohol	0.5g
3.	Hard paraffin	0.5g
4.	Soft paraffin	8.5g

Table 6: Formulation of Herbal ointment

Formulation	extract (g)	Ointment base q.s. (g)
F1	0.5	10
F2	1	10
F3	1.5	10

Evaluation of ointment

The prepared ointment was assessed using the parameters such as spreadability, irritancy effect, pH, etc.

- **Physical evaluation:** Physical parameters of he prepared ointment was assessed using the parameters such as spreadability, irritancy effect, pH, etc.
- **Consistency:** It was found to be smooth with no greediness observed.
- **Non irritancy test:** It was carried out by applying the prepared herbal formulation to the skin of humans and observing for the effect.
- **Solubility:** Solubility was treated for prepared ointment where it was found to be soluble in boiling water, miscible with ethanol, ether, chloroform.

Determination of PH

For measuring the pH of the ointment, a digital pH meter was calibrated. To prepare and check the pH of samples, 0.5g of each ointment was dissolved in 50 mL of distilled water and allowed to sit for 2 hours. The pH meter was standardized with the appropriate buffer solutions. Each sample solution was measured in triplicate, and the average values were recorded[27].

Spreadability

Spreadability refers to product that can be spread the ease. It is generally considered as a desirable characteristic of ointments. Without too much dragging, the cream base should spread easily and during rubbing it should not generate greater friction. To obtain a uniform thickness ointment was sandwiched between the two glass slides, and a weight (100 g) was placed on the glass slide for 5 min to compress the sample. A weight of 250 g was added to the pan. The time required before the two slides could be separated was considered a measurement of spreadability [28].

Homogeneity

By visual inspection based on their appearance all the developed ointments were tested for homogeneity [29].

Tube extrudability

It is a common test to obtain the force required to extrude the substance from the tube. The method applied for finding the applied shear in the region of rheogram corresponding to a shear rate exceeding the yield value and exhibiting consequent plug flow. In the present study, the method used for evaluating ointment formulation for extrudability external pressure was applied on the filling tube to evaluate extrudability according to the quantity, in percentage, of ointment extruded on a single application of pressure. The more amount of ointment extruded is considered to be a better extrudability. The ointment was filled into a clean lacquered aluminium collapsible tube that has a nozzle tip with a 5 mm opening and was extruded by applying external pressure with a finger. Extrudability was measured based on the amount of ointment extruded from the nozzle tip of the tube when an external pressure was applied to the tube [30].

Results

The phytochemical screening revealed the existence of major groups of phytochemicals, including alkaloids, flavonoids, and terpenoids. Physicochemical characteristics such as fluorescence analysis showed different illuminance when reacting in the presence of various reagents. HPTLC studies were performed by using CAMAG technique to detect the presence of flavonoids at 366 nm at which spots are visualized at an R_f value of 0.43,0.47,0.51 and 0.74 respectively. The extract was incorporated and formulated into herbal ointment and different parameters evaluated which are within acceptance range.

Discussion

This investigation concludes preliminary phytochemical screening indicated diverse phytochemical constituents like flavonoids, alkaloids, and terpenoids that may be responsible for the numerous health benefits of this herbal based medicine. The evidence suggests that the historically minimizing and reducing effects of herbal based medicines has led to a decline in synthetic compounds in favor of herbal-

based products. Therefore, the optimal formulating of these herbal products provides to greatest compatibilities for dose precision and the least variability in bulk content.

Acknowledgment: The authors are thankful to the authorities of Aditya College of Pharmacy in providing the necessary laboratory facilities which enabled for successful completion of the research work.

References

1. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4:177.
2. World Health Organization. Traditional medicine [Internet]. Available from: http://www.who.int/topics/traditional_medicine/en/
3. Li JWH, Vederas JC. Drug discovery and natural products: End of an era or an endless frontier. Science. 2009;325(5937):161-165.
4. Sahoo N, Manchikanti P, Dey S. Herbal drugs: Standards and regulation. Fitoterapia. 2010;81(6):462-471.
5. Richter RK. Herbal Medicine: Chaos in the Marketplace. New York: Haworth Herbal Press; 2003.
6. Rao GP, Baghel AKS, Singh RK, Chatterji KS. Antiviral activity of coralloid root of *Cycas revoluta* extract against some viruses of tomato plant. Experientia. 1984;40(11):1257-1258.
7. Duke JA, Ayensu ES. Medicinal Plants of China. 1st ed. Algonac (MI): Reference Publications; 1985.
8. Mourya MK, Prakash A, Swami A, Singh GK, Mathur A. Leaves of *Cycas revoluta*: potent antimicrobial and antioxidant agent. World J Sci. 2011;1(10):11-20.
9. Arshad M, Mumtaz MW, Chaudhary AR, Rashid U, Ali M, Mukhtar H, *et al.* Metabolite profiling of *Cycas revoluta* leaf extract and docking studies on alpha-glucosidase inhibitory molecular targets by phytochemicals. Pak J Pharm Sci. 2009;32(4):871-874.
10. Zazharskyi VV, Davydenko PO, Kulishenko OM, Borovik IV, Brygadyrenko VV. Antimicrobial activity of 50 plant extracts. Biosyst Divers. 2019;27(2):163-169.
11. Shahid W, Durrani R, Iram S, Durrani M, Khan FA. Antibacterial activity *in vitro* of medicinal plants. Sky J Microbiol Res. 2013;1(2):5-21.
12. Moawad A, Hetta M, Zjawiony JK, Jacob MR, Hifnawy M, Marais JPJ, *et al.* Phytochemical investigation of Cycas circinalis and *Cycas revoluta* leaflets: moderately active antibacterial biflavonoids. Planta Med. 2010;76(8):796-802.
13. Negm WA, Ibrahim ARS, Abo El-Seoud K, Attia GI, Ragab AE. A new cytotoxic and antioxidant amentoflavone monoglucoside from *Cycas revoluta* Thunb growing in Egypt. J Pharm Sci Res. 2016;8(5):343-350.
14. Bera S, Das B, De A, Barua A, Das S, De B, *et al.* Metabolite profiling and *in vitro* colon cancer protective activity of *Cycas revoluta* cone extract. Nat Prod Res. 2018;32(24):1-5.
15. Duke JA, Ayensu ES. Medicinal Plants of China. 1st ed. Algonac (MI): Reference Publications; 1985.
16. Moawad A, Hetta M, Zjawiony JK, Jacob MR, Hifnawy M, Ferreira D. Phytochemical investigation of C. circinalis and C. revoluta. Planta Med. 2010;76(8):796-802.
17. Moawad A, Hetta M, Zjawiony JK, Ferreira D, Hifnawy M. Two new dihydroamentoflavone glycosides from *Cycas revoluta*. Nat Prod Res. 2014;28(1):41-7.
18. Varshney AK, Mah T, Khan NU, Rahman W, Hwa CW, Okigawa M, *et al.* Biflavonoids from *Cycas revoluta*, C. circinalis and C. rumphii. Indian J Chem. 1973;11:1209-1214.
19. Shah BS, Quadry JS. Textbook of Pharmacognosy. 23rd ed. India: B.S. Prakashan; 1980. p.16, 24.
20. Kokate CK. Practical Pharmacognosy. 2nd ed. New Delhi: Vallabh Prakashan; 2002. p.111-113.
21. Gupta MK, Sharma PK, Ansari SH, Lagarkha R. Pharmacognostical evaluation of Grewia asiatica fruits. Int J Plant Sci. 2006;1(2):249-251.
22. Kokashi CJ, Kokashi RJ, Sharma M. Fluorescence of powdered vegetable drugs in ultraviolet radiation. J Am Pharm Assoc. 1958;47(10):715-717.
23. Pathan A, Bond J, Gaskin R. Sample preparation for scanning electron microscopy of plant surfaces horses for courses. Micron. 2008;39(8):1049-1061.
24. Wulandari L, Yuni E, *et al.* Analysis of flavonoid in medicinal plant extract using infrared spectroscopy and chemometrics. J Chem Pharm Res. 2016 Jul 26.
25. Pharmacognosy Network. Infrared spectroscopy [Internet]. Available from: https://pharmacognosy.in/tag/infrared-spectroscopy
26. Chhetri HP, Yogol NS, Sherchan J, KC A, Mansoor S, Thapa P. Formulation and evaluation of antimicrobial herbal ointment. Kathmandu Univ J Sci Eng Technol. 2010;6(1):102-107.
27. Ravindra TJ, Pratibha RP, Payal HP. Formulation and evaluation of semisolid preparations (ointment, gel and cream) of thiocolchicoside. J Pharm Biomed Sci. 2011;8:1-6.
28. Sabale V, Kunjwani H, Sabale P. Formulation and *in vitro* evaluation of the topical antiaging preparation of the fruit of Benincasa hispida. J Ayurveda Integr Med. 2011;2(3):124-129.
29. Dey S, Mazumder B, Patel JR. Enhanced percutaneous permeability of acyclovir by DMSO from topical gel formulation. Int J Pharm Sci Drug Res. 2009;2(4):384-391.
30. Rajasree PH, Viswanad V. Formulation and evaluation of antiseptic activity of polyherbal ointment. Int J Pharm Life Sci. 2012;3(10):2021-2031.