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PHYTOCHEMICAL SCREENING OF *Nelumbo nucifera* Gaertn. RHIZOME BY HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

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ABSTRACT

The presence of phytochemical compounds in plants indicate its medicinal potential which plays a significant role to develop it as a therapeutic agent. *N.nucifera* commonly known as Lotus and Kamala in Hindi, is widely used in traditional and folklore medicine. This plant is reported to have various secondary metabolites, which is having several medicinal uses. HPTLC has become the most sophisticated analytical tool for the detection of phytocompounds present in plants due to its simplicity, reproducibility and reliability. In the present study, a pioneering attempt has been made to focus on HPTLC phytochemical screening of *Nelumbo nucifera* Gaertn. rhizome for the presence of thirteen different secondary metabolites. Results of this phytochemical screening revealed the presence of secondary metabolites like bitter drugs, essential oils, flavonoids, saponins, triterpenes, etc. in *Nelumbo nucifera* Gaertn. rhizome.

Keywords: *Nelumbo nucifera*, HPTLC, Secondary metabolites, Phytochemical profiling, Rhizome

INTRODUCTION

Nowadays, people have more faith toward herbal medicines than modern medicines due to occurrence of many side effects of modern medicines **[1]**.

Herbal medicines are complex phytochemical mixtures obtained from plant sources which are widely used in the management of healthcare system in both developed and developing countries **[2]**. Herbal medicines are considered as the potential source for novel bioactive molecules due to their potent therapeutic efficacy, no side effects and economic viability **[3]**. Cragg and Newman (2013) reported that only 6% of existing plant species have been systematically investigated pharmacologically and only around 15% phytochemically **[4]**.

The rich biodiversity of plants makes them a treasure house for obtaining new and novel compounds either themselves as drugs or precursor for the synthesis of new chemical substances which can be used in drug **[5]**. Herbal drugs and its formulations are used to standardized on the basis of the presence of known active ingredient. This will facilitate in establishing the drug's quality, depending on the characteristic phytochemical profile of it as plants contain a large number of active substances **[1]**.

Medicinal plants contain several different phytochemicals that may act individually, additively or in synergy to improve health **[5]**. They are synthesized by specific biochemical pathways, for plant defense and adaptation to environmental stress. These bioactive compounds are generally accumulated as secondary metabolites inplant cells but their concentration varies according to the plant part. Many of the secondary metabolites isolated from plants are used in pharmaceutical drug industry. Such screening for phytochemicals has been done in many plants **[6]**.

The therapeutic efficacy of plants is because of these compounds which include alkaloids, flavonoids, saponins, terpenoids, steroids, phlobatannins, glycosides, tannins, etc. All these secondary metabolites are known for curing one or other diseases and are used in both traditional and modern medicine **[7]**. The presence of tannins shows plant possess anti-parasitic, antiviral and antibacterial activities. Flavonoids are the phenolic compounds having antioxidant, anti-inflammatory, anti-allergic and anticancer activities. Saponins acts as anti-feedants and used as adjuvant in vaccines. Phenols are used as antiseptic and active ingredient in some oral analgesics **[8]**. Knowing the phytochemical profile of different plant parts is desirable so that one can decide the part to be explored for any particular activity and it can also help one to decide the part(s) to be chosen for any synergistic evaluation **[9]**.

Due to the natural variability and higher demand of herbal medicines, chemical analysis of plant materials is of great challenge and requires a special approach. However, among the various analytical approaches, chromatography is often considered to be the most appropriate one to use **[10]**. Among different chromatographic methods available, HPTLC has become the most potent tool for the analysis of wide-range of compounds both efficiently and cost effectively **[11, 12]**.

Unlike other analytical methods, HPTLC produces visible chromatograms complex information about the entire sample. The advantages of automation, scanning, full optimization, selective detection principle, minimum sample preparation and hyphenation enable HPTLC as one of the most powerful analytical techniques for chromatographic information of complex mixtures of pharmaceuticals, natural products, herbal formulations, etc. **[2]**. Additionally, it can handle several samples of even divergent nature and composition. It gives better resolution and assessment of active components with minimum troubleshooting in lesser time **[11, 12]**.

Nelumbo nucifera Gaertn (Family: Nymphaeaceae) is a large aquatic herb with stout, creeping and yellowish white rhizomes **[13]**. It is a well-known plant and commonly known by names like lotus and sacred lotus. It is commonly called as Kamala or Padma in India **[14]**. There are two forms-one with white flowers commonly called Pundarika or sveta kamala and the other with pink or reddish pink flowers called Rakta Kamala **[13]**. *N. nucifera* is a native of China, Japan and India. Mainly it is cultivated in China and Japan in terraced fields for its edible rhizomes and seeds **[15]**.

Almost all parts of *Nelumbo nucifera* Gaertnare used in indigenous system of medicine **[16]**. Rhizome commonly called as 'Kamalakand' are consumed as food in Asian countries. They are nutritive, mucillginous, diuretic, collagogue, demulcent **[17]**.

Nelumbo nucifera rhizomes have high economic value due to the abundance of bioactive compounds that have a miraculous health promoting effect on the human body. The rhizome of *Nelumbo nucifera* has medicinal importance, such as used in the treatment of dysentery, as a demulcent, cholagogue, nutritive, diuretic and in the treatment of dyspepsia, diarrhea and piles **[17, 18]**. It possesses various pharmacological activities *viz*. anti-diarrheal, antipyretic, antimicrobial, antidiarrheal, diuretic, anti-inflamma-

tory, immunomodulatory and psycho-pharmacological activities **[19]**.

Thus, the aim of the present research work is to explore detailed phytochemical screening of *Nelumbo nucifera* Gaertn. rhizome using HPTLC technique which may lead to confirm the presence of phytochemicals responsible for its pharmacological activities.

MATERIALS AND METHOD

Plant material- Collection and Authentication

The rhizomes of *Nelumbo nucifera* Gaertn. were collected from a pond, Badlapur (Maharashtra, India) and authenticated from Agharkar Research Institute, Pune (Maharashtra, India). The rhizomes were washed thoroughly with water to remove mud, cut into slices with a knife and dried in preset oven at 40 ± 2˚C for about four days, ground into powder and stored in airtight container for further analysis.

Chemicals and Reagents

All the chemicals and reagents used were of analytical grade and purchased from Sigma-Aldrich Chemical Co., Mumbai. The precoated TLC silica gel $60F_{254}$ plates were obtained from E. Merck (India).

Sample Preparation

Samples of *Nelumbo nucifera* Gaertn. rhizome was prepared differently for various secondary metabolites according to standard method described by Wagner and Bladt (1984) **[20]**. All the samples were filtered through Whatman filter paper no. 1 before HPTLC analysis.

High- performance thin- layer (HPTLC) Chromatography analysis:

Instrumentation and operating conditions

Analysis work was carried out on HPTLC equipment, CAMAG made (Muttenz, Switzerland) which consist of Linomat-V sample applicator fitted with a 100 µL syringe (Hamilton, Switzerland), CAMAG TLC visualizer, CAMAG TLC Scanner 3 and WinCATS software. Analysis was performed by using TLC precoated silica gel 60 F_{254} aluminium plates with 200 µm thickness (E. Merck, Mumbai, India). 10 µl of samples were applied to the plate using the Linomat-V sample applicator fitted with a 100 µL syringe. After the application, plates were developed in CAMAG twin-trough glass chamber presaturated for 20 mins at room temperature, with respective mobile phase. The TLC plates were developed up to the distance of 8 cm. After development, plates were dried at room temperature, derivatized with respective derivatizing reagents for 30 seconds. Mobile phases and derivatizing agents for respective

secondary metabolites are mentioned in **Table 1**. Plates were air dried and heated on HPTLC Plate heater at 110 °C for 10 mins. The plate was kept in CAMAG TLC visualizer and the images were captured. Densitometric scanning was then performed at 254nm, 366 nm and visible light using CAMAG TLC Scanner 3 with winCATS software version 1.4.6.The slit dimension of 6.0 × 0.45 mm with scanning speed of 20 mm/sec was used throughout the analysis.

Table 1: Mobile phases and Derivatizing agents for respective secondary metabolites

Sr. No.	Phytoconstituents	Mobile phases	Derivatizing agents	Observation
1.	Alkaloid Drugs	Toluene: Ethyl Acetate: Methanol: Ammonia (30:30:15:1)	Dragendorff's reagent	Brown zones appear immediately after derivatization
2.	Anthracene Derivatives	Ethyl acetate:Methanol: Water (81:11:8)	Potassium hydroxide reagent	Yellow or red-brown fluorescence at 366nm
3.	Bitter Drugs	Ethyl acetate: Methanol: Water (77:15:8)	Anisaldehyde Sulphuric acid reagent	Red-violet, brown, blue green, blue, grey bands under visible light
4.	Cardiac Glycosides	Ethyl acetate: Methanol: Water (81:11:8)	Sulphuric acid reagent	Yellow, brown and blue zones under visible light
5.	Coumarin Drugs	Toluene: Ether (1:1saturated with 10% acetic acid) lower phase	Potassium hydroxide reagent	Blue, blue green and yellow fluorescence under 366 nm
6.	Essential Oils	Toluene: Ethyl acetate (93:7)	Anisaldehyde Sulphuricacid reagent	Blue, red, green and brown coloration in visible light
7.	Flavonoids	Ethyl acetate: Formic acid: glacial acetic acid: water (10:0.5:0.5:1.3)	Sulphuric acid reagent	Blue, green and red fluorescence under UV-366 nm
8.	Lignans	Toluene: Ethyl acetate (70:30)	Sulphuric acid reagent	Blue fluorescence at 366 nm
9.	Pungent -Tasting Principles	Toluene: Ethyl acetate (70:30)	Vanillin –Sulphuric acid reagent	Lemon yellow and blue to violet bands in visible light
10.	Saponins	Chloroform: Glacial acetic acid: Methanol: Water (64:32:12:8)	Vanillin – Sulphuric acid reagent	Blue, blue-violet and yellow brown zones in visible light
11.	Triterpenes	Ethyl acetate: Glacial acetic acid: Formic acid: Water (90:3:3:2)	Anisaldehyde Sulphuric acid reagent	Blue violet dumbbell shape quenching under 366 nm
12.	Valepotriates	Toluene: Ethyl acetate (75:25)	Anisaldehyde Sulphuric acid reagent	A violet and blue zone in visible light

RESULTS AND DISCUSSION

The medicinal significance of any plant is due to the presence of various bioactive phytocompounds present in some or all of its parts. These compounds generate characteristic physiological action on humans and help to treat various ailments **[21]**. Therefore, detection of these phytocompounds in any plant is desirable. HPTLC is simple, fast and precise method for a better identification of the bioactive compounds **[8]**.

In the present study, rhizome extracts of *Nelumbo nucifera* Gaertn. were evaluated for the detection of twelve secondary metabolites namely alkaloid drugs, anthracene derivatives, bitter drugs, cardiac glycosides, coumarin drugs, essential oils, flavonoids, lignans, pungent-tasting principles, saponins, triterpenes and valepotriates by HPTLC. This study revealed that the solvent system developed and the specific derivatizing agents used gave well-resolved bands for secondary metabolites present in the rhizome extract of *Nelumbo nucifera* Gaertn.

The results on presence and absence of secondary metabolites in rhizome extract are depicted in **Table 2**. The chemo profile of *Nelumbo nucifer*a Gaertn. rhizome extract provided a set of peaks with Rf values and their corresponding area percentage which indicates the presence of specific chemical compounds. The Rf values of respective compounds in all the chromatograms are given in **Table 3**. The developed chromatograms for respective secondary phytocompounds of rhizome are presented in **Figure 1**.

Table 2: Detection of secondary metabolites in *Nelumbo nucifera* Gaertn. *rhizome* by HPTLC

Sr. No.	Phytoconstituents	Present/ Absent
1.	Alkaloid Drugs	ND
2.	Anthracene Derivatives	D
3.	Bitter Drugs	D
4.	Cardiac Glycosides	D
5.	Coumarin Drugs	D
6.	Essential Oils	D
7.	Flavonoids	D
8.	Lignans	D
9.	Pungent –Tasting Principles	D
10.	Saponins	D
11.	Triterpenes	D
12.	Valepotriates	D

Keywords: 'ND'–Not detected; 'D'-Detected

Table 3: Retention factor (R_f) of secondary metabolites in *Nelumbo nucifera* Gaertn. *rhizome* by HPTLC

Sr. No.	Phytoconstituents	R_f values
1.	Alkaloid Drugs	-
2.	Anthracene Derivatives	0.27, 0.33, 0.49, 0.59, 0.65, 0.83, 0.88
3.	Bitter Drugs	0.28, 0.35, 0.41, 0.63, 0.86
4.	Cardiac Glycosides	0.27,0.32, 0.37, 0.41, 0.49, 0.52, 0.61, 0.68, 0.75, 0.81
5.	Coumarin Drugs	0.20, 0.36, 0.50, 0.73, 0.89
6.	Essential Oils	0.34, 0.47, 0.66, 0.73, 0.79
7.	Flavonoids	0.17, 0.19, 0.25, 0.38,0.51,0.77
8.	Lignans	0.30, 0.41, 0.51, 0.56, 0.67, 0.85
9.	Pungent -Tasting Principles	0.13, 0.16, 0.21, 0.27, 0.41, 0.67, 0.82
10.	Saponins	0.15, 0.20, 0.36, 0.39, 0.55, 0.76, 0.83, 0.85
11.	Triterpenes	0.19, 0.27, 0.38, 0.51, 0.63, 0.68,0.74
12.	Valepotriates	0.14, 0.20, 0.27, 0.34, 0.37, 0.43, 0.57, 0.67, 0.74, 0.85

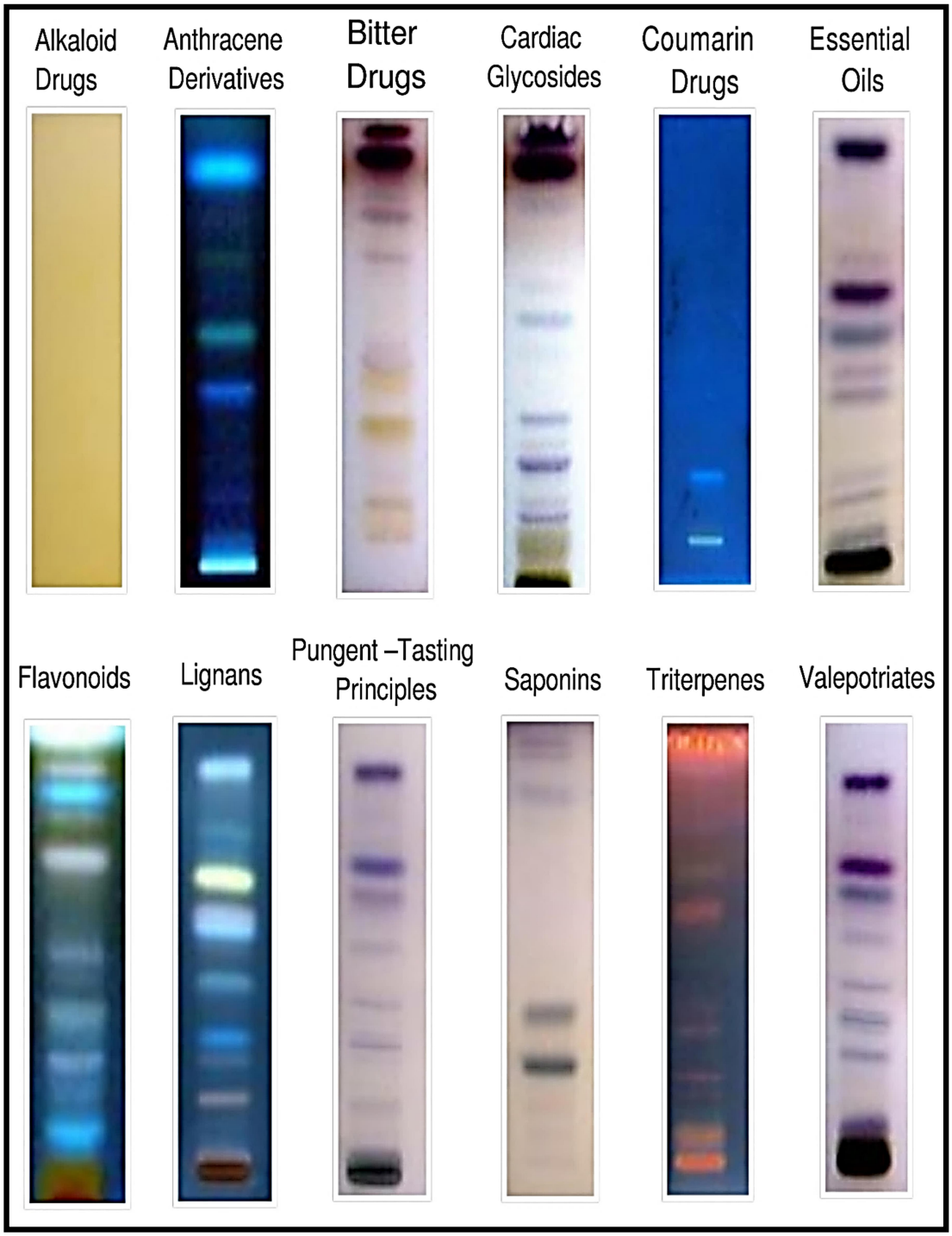

Figure 1: HPTLC chromatograms of secondary metabolites for *Nelumbo nucifera* Gaertn. *rhizome*

CONCLUSION

In the 21st century, the development of phytochemical profile of medicinal plants have been considered as a search of promising drug for the management of health care. All plants synthesize phytochemicals, which are active ingredients and possess therapeutic

properties that are considered as a medicine or drug. Present study confirms the presence of many important phytochemicals in the *Nelumbo nucifera* Gaertn**.** rhizome, hence it can be seen as potential source of novel beneficial drugs. Further work to characterize the other chemical constituents and perform quantitative estimation with marker compounds is yet necessary.

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REFERENCES

[1] Mukherjee PK, Banerjee S, Kar A, Chanda J, Drugs from Our Ancestors: Tradition to Innovation (In) Herbal Medicine in India-Indigenous Knowledge, Practice, Innovation and its Value, Sen S, Chakraborty R (Eds), Springer Singapore, 2020, 263-279.

[2] Lalhriatpuii T, HPTLC Fingerprint in Herbal Drug Formulations. In: Sen S., Chakraborty R. (eds) Herbal Medicine in India. Springer, Singapore, 2020.

[3] Shakya AK, Medicinal plants: Future source of new drugs, International journal of herbal medicine, 4(4), 2016, 59–64.

[4] Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. Biochim. Biophys. Acta., 1830, 2013, 3670–3695.

[5] Shwetha C, Latha KP, Pushpa B, Shruthi A, Vaidya VP, Phytochemical screening and evaluation of in vitro antioxidant activity, Total phenolics and Total flavanoids of *Holarrhena anti dysentrica* leaf extracts. International Journal of Research in Pharmacy and Chemistry, 1, 2011, 546-550.

[6] Angamuthu J, Ganapathy M, Evanjelene VK, Ayyavuv N, Padamanabhan V, Evaluation of phytochemical analysis and antimicrobial activity of leaf and fruit extract of *Physalis minima.* Int J Emerg Technol Adv Eng., 4(1), 2014, 462-465.

[7] Rehab A, Hussein and Amira A, El-Anssary, Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants, Herbal Medicine,

Philip F. Builders, IntechOpen, 2018.

[8] Mehta S, Singh RP, Saklani P, Phytochemical screening and TLC profiling of various extracts of *Reinwardtia indica.* International Journal of Pharmacognosy and Phytochemical Research, 9(4), 2017, 523-527.

[9] Naima F, Houcine B, Houcine A, Nawal O, Screening of Some Plant Materials Used in South-West Algerian Traditional Medicine for Their Antibacterial Activity. International Journal of Phyto-medicine, 9(4), 2017, 619-627.

[10] Kokate CK, Purohit AP, Gokhale SB, Pharmacognosy, 42th ed., Nirali Publication, Pune, 2008.

[11] Eike R, Anne S,High performance Thin-Layer Chromatography for the Analysis of Medicinal Plants, Thieme medical publisher New York, 2006.

[12] Jain A, Parashar AK, Nema RK, Narsinghani T.High Performance Thin Layer Chromatography (HPTLC): A Modern Analytical Tool for Chemical Analysis. Current Research in Pharmaceutical Sciences, 04 (01), 2014, 08-14.

[13] Chopra RN, Chopra IC, Handa KL, Kapur LD, Indigenous drugs of India, 2nd Ed, U.N. Dhur and sons Pvt. Limited, Calcutta, 1958, 679.

[14] Kaur P, Kaur L, Kaur N, Singh A, Kaur J, Kaur H, Kaur N, Kaur M, A brief review on pharmaceutical uses of *Nelumbo nucifera*. Journal of Pharmacognosy and Phyto-chemistry, 8(3), 2019, 3966-3972.

[15] Anonymous, The Wealth of India, Vol III, P.ID., CSIR, New Delhi, 1966.

[16] Raghunathan K, Mitra R, Pharmacognosy of indigenous Drugs, C.C.R.A.S., New Delhi, Vol. 11, 1982, 206.

[17] Kirtikar KR, Basu BD, Indian Medicinal Plants, 2nd Ed, International Book Distributors, New Delhi, 1975, 116–120.

[18] Chatterjee A, Pakrashi SC, The Treatise on Indian Medicinal Plants, vol. 1. Publication and Information Directorate, New Delhi, 1991, 94–96.

[19] Ahmed H, Hakani G, Aslam M, Khatian N,A review of the important pharmacological activities of *Nelumbo nucifera*: A prodigious rhizome. International Journal of Biomedical and Advance Research, 10(01), 2019, 1-7.

[20] Wagner H, Bladt S, Plant Drug Analysis, A thin layer chromatography atlas, Springer –

Verlag Berlin Heidenberg New York Yokyo, 1984.

[21] Akinmoladunm AC, Ibukun EO, Afor E, Obutor EM, Farombi EO, Phytochemical constituent and antioxidant activity of extract from leaves of *Ocimum gratissimum*. Science Research Assay, 2, 2007, 163-166.