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VALIDATING THE PROPERTIES OF *NYMPHAEA PUBESCENS* WILLD. BY PERFORMING UV-VIS, TLC, FTIR AND HPLC ANALYSES

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ABSTRACT: Introduction: *Nymphaea pubescens* Willd. is one of the well-known medicinal plants, being used from ancient times in India and commonly called "Kumuda". It is an aquatic perennial herb. It has leaves having a smooth upper side, a strong root, and a branched rhizome. According to Bentham and Hooker classification "Genera Plantarum", this plant belongs to the division Polypetale of Dicotyledones, which has been divided into 15 order and 84 families. Nymphaea comes under the Araceae family, which itself contains 8 genera and 100 species. **Method:** Morphological characters have been examined through a transverse section cut by microtome. Physiochemical studies and other screenings were performed accordingly. In addition to this, UV-VIS, TLC, FTIR, and HPLC tests were also carried out for further validation. **Result:** The morphological and physiochemical examination revealed the presence of saponins, proteins, and various other tests in *Nymphaea pubescens* Willd. **Conclusion:** The microscopic, UV-VIS, TLC, FTIR, and HPLC validation can be helpful for using it for medicinal and commercial purposes.

INTRODUCTION: *Nymphaea pubescens* Willd. is also known as water lily; it is grown in ponds, lakes, and water bodies, having simple heart-shaped leaves, branched rhizome, striated seed, and globular fruit [1]. The whole plant is used as a medicine in Type 2 diabetes mellitus and urinary tract disease [2]. Although it is one of the types of lotus species, a very brief illustration is found in different botanical and Ayurvedic treatises. The scarcity of information regarding the characteristics and its uses motivates the author to review it extensively in various research journals and other related literature.

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And to standardized the parameter after performing Organoleptic properties, powder microscopy, physiochemical, UV-VIS, TLC, FTIR, and HPLC studies. Such exploration and validation help society in the treatment of various diseases and for medicinal and commercial purposes.

MATERIALS AND METHODS:

Collection: *Nymphaea pubescens* Willd. was collected from its natural habitat near Banaras Hindu University, Varanasi, in July 2018.

Authentication of Plants: Sample (Voucher specimen no. Nymphaea.2018/1) was authenticated by the expert from the Department of Botany, Institute of Science, Banaras Hindu University, Varanasi. Plant specimen was deposited in the museum of the Department of Dravyaguna, Faculty of Ayurveda, for future reference.

Chemicals: All analytical grade chemicals used in the study were purchased through Advanced Quality traders, E. Merk, Germany.

Organoleptic Evaluation: Organoleptic evaluation of the plant sample was done with the help of sensory organs [3].

Powder Microscopy: The coarse powder of the sample was boiled with chloral hydrate and collected in a Petri dish, and stained with the different reagent [4].

Physiochemical Study: Coarse powder of the sample was used for an analytical test which is shown in table number [3].

Loss on Drying: About 10 gm of the sample was placed in a tarred evaporating dish, and the sample was dried at 105 ºC for 5 h and weighed. After that, it was cooled in desiccators for 30 min and weighed.

Determination of Total Ash: About 2.0 g of the sample was incinerated in a tarred silica dish at a temperature not exceeding 450 ºC until free carbon was left, cooled, and finally weighed.

Acid Insoluble Ash: Ash was dissolved in 25 ml of dilute HCL and boiled for 5 min; then, insoluble matter was collected on an ash-less filter paper, washed with hot water, and ignited to constant weight.

Water Soluble Ash: The sample was dissolved in 25 ml of diluted HCl and made up of 50 ml with water and boiled. Then filtered with Whatmann filter paper and residue was collected.

It was weighed and maintained in a muffle furnace for 6 h at 450-650 ºC. The crucible was taken out and cooled at room temperature and weighed.

UV-Vis (Ultraviolet-Visible Spectroscopy): 1 gram of plant extracts added in 10 ml of distill water then filtered with the help of cartilage (0.2 µm) afterward, it was scanned under ultraviolet, visible spectrophotometer (Perkin Elmer) at a range of 200-900 nm to measuring the size of biomolecules and uncertainty source that may arise from nature of the compound of plant extract.

TLC (Thin Layer Chromatography): Thin layer chromatography was detected by observation of spots for identical R_f values and to determine the purity of a sample.

Stationary Phase: Pre-coated plate with silica gel G (Merck) was used as the stationary phase.

Solvent System: Toluene: Ethyl acetate: Formic acid: Methanol was used as a solvent system in the respective ratio of 4.3: 4.3: 1.2: 0.3.

Spraying Reagent: Methanol: sulphuric acid (9:1) was used for drying plates in a hot air oven.

Procedure: Extract was applied 2 cm on the lower edge of the plate with the help of a microcapillary tube. And then extracts were loaded in a small volume spot on each plate, the plate was taken out, the solvent front was marked, and the plate was dried at room temperature.

FTIR (Fourier- Transform Infrared Spectroscopy): A pinch of powder drug was taken and placed over the crystal present on stage. The IR spectrum (Perkin Elmer, Spectrum-2) was scanned between 4000 to 400-1, and transmittance was recorded.

Before scanning the sample, the background signal was also recorded. The peaks thus obtained were matched against the IR interpretation chart, and the functional groups were noted.

HPLC (High-Performance Liquid Chromatography): 1 g of plant extract is added in 10 ml of methanol then sonicator in the sonicator machine (Labman), afterward filtered with the help of cartilage (0.2 µm) and inject with micro syringe (20 µl) and finally scanned with HPLC machine to detect the flavonoids and Phenol.

Standard: (Flavonoids and Phenol) Catechin hydrate, Myricetin, Rutin, Quercetin, Caffeic acid, Kaempferol and Gallic acid, all solution is prepared in methanol (1 mg/ml).

Mobil Phase A: methanol: acetonitrile: water: acetic acid (50 ml : 25 ml : 425 ml : 5 ml) For 0-20 min.

Mobil Phase B: methanol: acetonitrile: acetic acid (300 ml : 200 ml : 5 ml) for 20- 25 min.

RESULTS:

Organoleptic Properties: Organoleptic properties of *N. pubescens* Willd. show is shown in **Table 1**.

TABLE 1: ORGANOLEPTIC PROPERTIES OF *N. PUBESCENS* WILLD.

Nature	Coarse powder
Size	Pass through 80 mm of mesh size sieve
Colour	Brownish black
Odour	Aromatic
Taste	Bitter
Texture	Rough

Powder Microscopy: Behaviour of *N. pubescens* Willd. powder with different reagents.

Physiochemical Study: Result of physiochemical analysis of *N. pubescens* Willd. is shown in **Table 3**. The method of loss on drying has actually calculated the presence of moister of *N. pubescence* (11.98 %) Less number of moister prevent from bacteria and fungus [5].

The total Ash value of N. pubescence (6.15%), indicates the presence of minerals in seed [6]. Acid insoluble ash of *N. pubescence* (11.8%) indicates the presence of the earth mineral and silica. 6.3% Water-soluble Ash presence in *N. pubescence* **Table 3**.

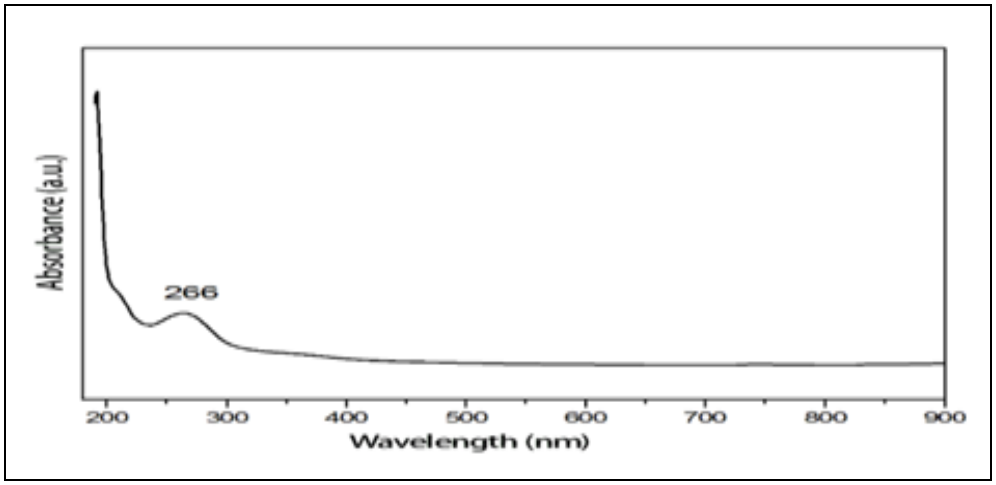
TABLE 2: POWDER MICROSCOPY OF *N. PUBESCENS*

Reagent	Observation	Characteristic
Powder + Ruthenium red	Black colour	Mucilaginous cells are absent
Powder + Sudan red	Pink colour	Cuticle is present
Powder + Dil. HCL	Soluble	Calcium oxalate crystals are present
Powder + Safranin	Red colour	Vesicle present
Powder + Phloroglucinol + Conc. HCl	Pink colour	Lignified cells are present
Powder + Dilute iodine solution + Conc. Sulphuric acid	Black colour	Hemicellulose absent
Powder + Sulphuric acid	Brown colour	Stone cell absent
Powder + Dilute iodine solution	No blue colour	Endodermis without starch

TABLE 3: PHYSIOCHEMICAL STUDY OF *N. PUBESCENS* WILLD.

Name of study	Result (%)
Loss on Drying	11.98
Determination of total ash	6.15
Acid Insoluble Ash	11.8
Water-soluble Ash	6.3

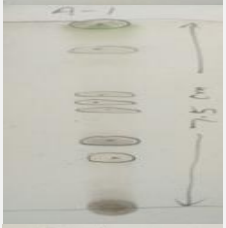
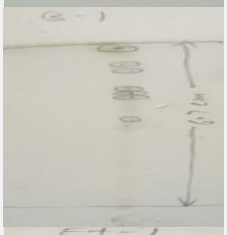
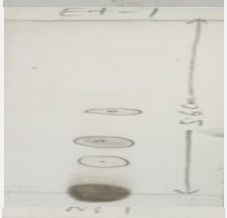
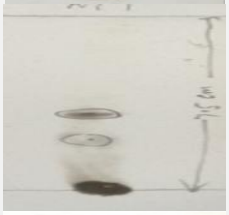
The Ultraviolet, visible spectroscopy profile of graph 1 of the extract was observed at 200-900 nm wavelength range and 266 nm recorded band. Phenolic and flavonoid components generally absorb at 230-290 nm [7]. Hence, it conform phenolic and flavonoids are present in the extract of *N. pubescens* Willd.



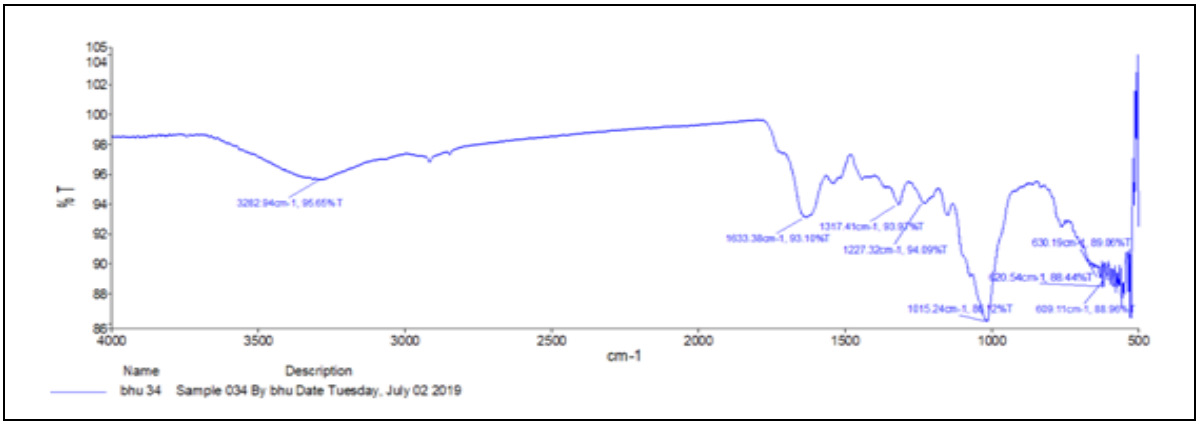
GRAPH 1: ULTRAVIOLET VISIBLE SPECTROSCOPY OF DISTIL WATER EXTRACT OF *N. PUBESCENS* WILLD

Thin Layer Chromatography: Thin layer chromatography was detected by the observation of spots for identical R_f values and to determine the purity of a sample. In petroleum ether extract showed six spot, Chloroform indicate five spot, Acetone and Benzene identified seven spots, Ethanol has three spots and lastly Methanol and distill water separate two spot **Table 4**. Hence, the TLC profile shows compounds are smoothly separated with the help of mobile phase 8 9 10 11.

TABLE 4: TLC OF *N. PUBESCENS* WILLD

Extract	Solvent Front (cm)	TLC plates	Peaks Obtained (cm)	R_f Value (cm)
Petroleum Ether	7.3		S_1 1.4	0.30
			S_2 2.7	0.36
			S_3 3.1	0.42
			S_4 3.9	0.53
			S_5 5.6	0.76
			S_6 6.7	0.91
Chloroform	7.5		S_1 2.9	0.38
			S_2 3.6	0.48
			S_3 4.6	0.61
			S_4 5	0.66
			S_5 7.1	0.94
Acetone	7.5		S_1 2	0.26
			S_2 2.8	0.37
			S_3 4	0.53
			S_4 4.3	0.55
			S_5 4.6	0.61
			S_6 6.4	0.85
			S_7 7.4	0.98
Benzene	6.7		S_1 3.5	0.52
			S_2 4.4	0.65
			S_3 4.6	0.68
			S_4 4.8	0.71
			S_5 5.5	0.82
			S_6 5.8	0.86
			S_7 6.5	0.97
Ethanol	5.6		S_1 1.4	0.25
			S_2 2.3	0.41
			S_3 3.6	0.64
Methanol	7.5		S_1 2.2	0.29
			S_2 3.3	0.44
Distil Water	7		S_1 1.2	0.17
			S_2 2	0.28

FTIR (Fourier- Transform Infrared Spectroscopy): FTIR is a characterization method who give the vibration energy based on peak value, compressing act of the functional group that is available on the extract of *N. pubescence*. The major bands were observed at 3282, 1633, 1317, 1227, 1015 and 630 to 609, cm^{-1}. The peak indicates **Table 5** OH stretching might be alcohol, carboxylic acid. CH stretching is alkyne. NH bending is an amine. C=C Stretching is Con. And conjugate alkene. OH, bending is Phenol. S=O Stretching is Sulfone. C-F Stretching is Fluro compound. C-N Stretching is Amine and Aromatic Amine. C-O Stretching is Alkyle arile ether, and C-X (X=Cl or Br) is halo compound [12, 13].

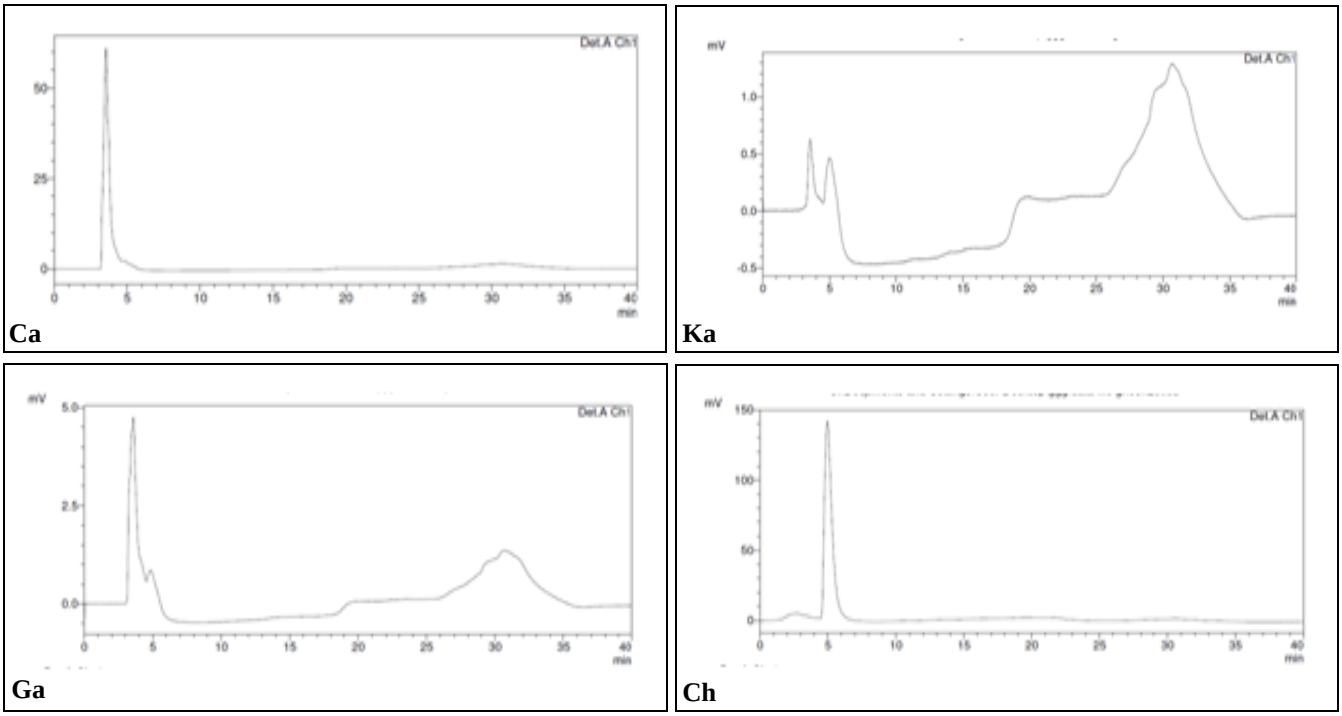


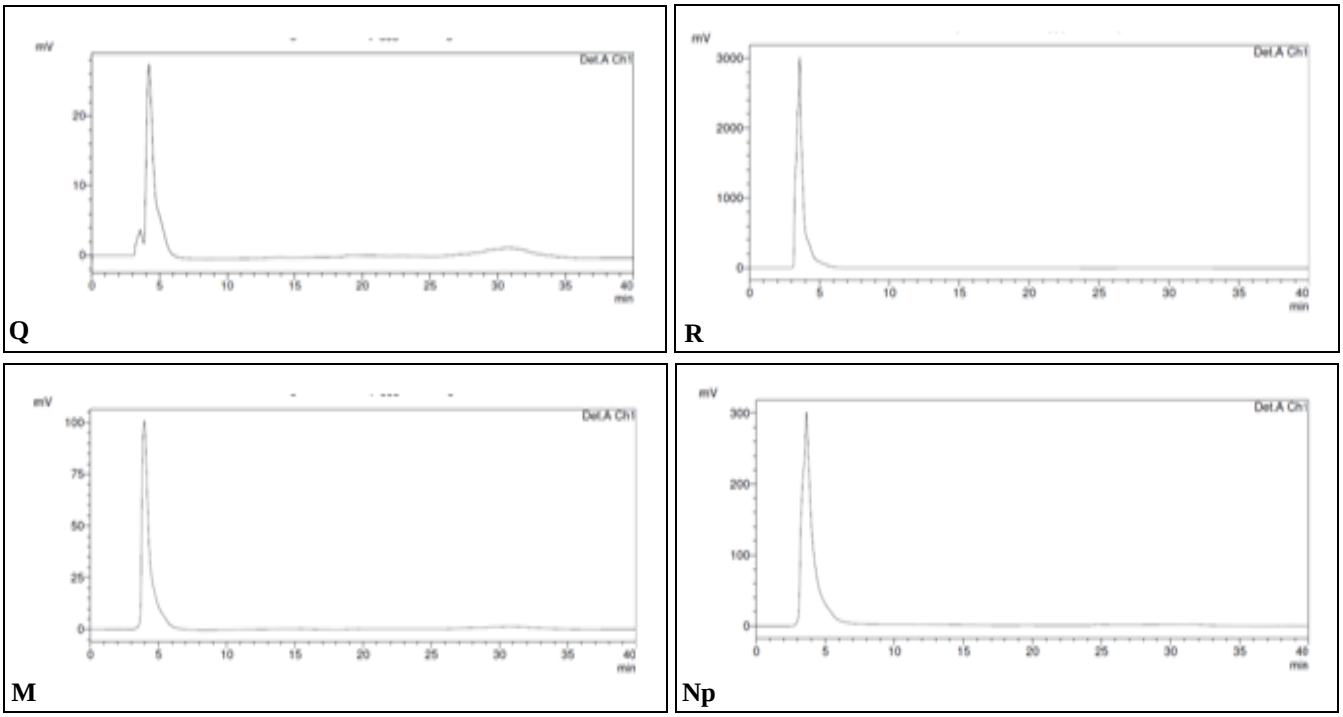
GRAPH 2: FTIR OF CRUDE EXTRACT OF *N. PUBESCENS* WILLD. Fourier transform infrared spectroscopy is a technique to measure the wavelength of the sample (*N. pubescens* Willd.) and find out the probable functional group

TABLE 5: FTIR OF *N. PUBESCENS* WILLD

FTIR Profile			
Peak Number	**X(cm-1)**	**Group**	**Compound Class**
1	3282.94	OH Stretching	Carboxylic acid, Alcohol
		CH Stretching	Alkyne
2	1633.38	C=C Stretching	Con. alkene
		N-H bending	Amine
		C=C Stretching	Conjugated alkene
3	1317.41	O-H bending	Phenol
		S=O Stretching	Sulfone
		C-N Stretching	Aromatic Amine
4	1227.32	C-F Stretching	Fluoro compound
		C-o Stretching	Alkyl aryl ether
		C-N Stretching	Amine
5	1015.24	C-F Stretching	Fluoro compound
6	630.19-609.11	C-X Stretching (X=Cl or Br)	Fluoro compound, Halo compound

HPLC (High Performance Liquid Chromatography): High-performance liquid chromatography of methanol extract of *N. Pubescens* Willd is shown in **Graph 3**.





GRAPH 3: HPLC CHROMATOGRAM OF STANDARD PHENOLIC ACID (Ca = Caffeic acid, K = Kaempferol, Ga = Gallic acid), Flavonoids (Ch = Catechin hydrate, Q = Quercetin, R = Rutin and M= Myricetin) and Np = methanol extract of Nymphaea pubescence

TABLE 6: ANALYTICAL CONDITIONS

Column	Shim-pack GIST/GISS C 18
Mobile Phase	Phase A- Methanol 10: Acetonitrile 5: Water 85: Acetic acid 1. Phase B- Methanol 60: Acetonitrile 40: Acetic acid 1.
Time Program	40 min
Flow rate	1 ml/min
Column Temp.	32 ºC
Injection Vol.	20 µL

TABLE 7: RETENTION TIME (RT), WAVELENGTH (NM), AREA AND HEIGHT OF METHANOL EXTRACT OF *N. PUBESCENCE* AND STANDARD OF PHENOLIC ACID AND FLAVONOIDS FOR HPLC METHOD VALIDATION

HPLC Profile				
Name of Extract and Standard	**λ_{max} (nm)**	**RT (min)**	**Area %**	**Height %**
Methanol extract	254	3.624	99.189	98.564
Caffeic acid	254	3.536	85.449	86.704
Kaempferol	254	4.971	34.333	22.269
Gallic acid	254	3.575	34.899	35.153
Catechin hydrate	254	4.944	88.978	91.905
Quercetin	254	4.218	90.996	86.989
Rutin	254	3.567	99.536	99.837
Myricetin	254	3.946	99.024	99.333

The HPLC machine details shown in **Table 6** and in **Table 7**. The plant extract was evaluated with seven standards (gallic acid, quercotin, catechin, rutin, caffic acid, myricetin) of phenolic and flavonoids phytoconstituents to detect their capability. Caffeic acid (3.536 RT), Gallic acid (3.575), and Rutin (3.567) are present in both extracts of methanol and distill water of N. pubescence. Rutin and Caffeic acid are both possessing in previous studies on another plant of *R. arvensis* [14].

CONCLUSION: The present study evaluates macroscopic, microscopic, powder microscopy, physiochemical, UV-VIS, FTIR, TLC, and HPLC of the whole plant of *N. Pubescens* Willd. for correct identification and standardization. Powder microscopy shows the presence of calcium oxalate crystal, lignified Cells, and cuticle. Physiochemical test showing 11.98% loss of the crude drug on drying, 6.15% of total ash, 11.8% of acid in soluble, 6.3% of water-insoluble ash.

The TLC result indicates that the mobile phase successfully separates the compound, and the R_f value shows the medicinal value of *N. Pubescens* Willd. FTIR shows the presence of different functional groups such as Carboxylic acid, Alcohol, Alkene, Amine, Conjugated alkene, Phenol, Sulfone, Aromatic Amine, Alkyl aryl ether, Fluoro compound, and Halo compound.

All of these functional groups have an anti-diabetic effect, especially, Tannin, flavonoids, and phenol are showing antioxidant properties as an explanation for anti-diabetic effects of the Nymphaea pubescence.

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