World Journal of Pharmaceutical Science and Research

www.wjpsronline.com

Research Article

ISSN: 2583-6579
SJIF Impact Factor: 6.916
Year - 2026
Volume: 5; Issue: 6
Page: 799-810

PHARMACOGNOSTIC STANDARDIZATION AND PHYTOCHEMICAL CHARACTERIZATION OF *PISTIA STRATIOTES* WITH EMPHASIS ON ITS ANTIOXIDANT POTENTIAL

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Article Received: 21 April 2026 | | Article Revised: 11 May 2026 | | Article Accepted: 01 June 2026

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DOI: https://doi.org/10.5281/zenodo.20688486

How to cite this Article: Rekha Nigam, Dr. Gaurav Mittal (2026) PHARMACOGNOSTIC STANDARDIZATION AND PHYTOCHEMICAL CHARACTERIZATION OF *PISTIA STRATIOTES* WITH EMPHASISONITS ANTIOXIDANT POTENTIAL. World Journal of Pharmaceutical Science and Research, 5(6), 799-810.

ABSTRACT

The present study evaluated the extractive values, phytochemical composition, quantitative constituents, and antioxidant activity of *Pistia stratiotes* leaf extracts using methanol and n-hexane as solvents. The extractive value results revealed that the methanolic extract exhibited a higher yield (17% w/w) compared to the n-hexane extract (9% w/w). This difference indicates that methanol, being a polar solvent, is more efficient in extracting a broader range of phytoconstituents from *Pistia stratiotes* leaves than the non-polar n-hexane. The higher yield also suggests the abundance of polar compounds in the plant material. Preliminary phytochemical screening confirmed the presence of several important bioactive compounds, including flavonoids, alkaloids, tannins, glycosides, phenols, and carbohydrates in both extracts. However, the methanolic extract showed comparatively better extraction of key phytochemicals such as saponins and glycosides, while n-hexane extract demonstrated stronger presence (+++) of flavonoids, tannins, and alkaloids. The absence of anthraquinones in both extracts indicates their negligible presence in the plant. Overall, the results suggest that solvent polarity plays a crucial role in determining the type and extent of phytochemical extraction. Quantitative analysis of the alcoholic (methanolic) leaf extract further supported the qualitative findings. Among the phytoconstituents, saponins were found in the highest concentration (2.42±0.56mg/ml), followed by phenols (1.44±0.055mg/ml). Alkaloids (0.058 ± 0.06 mg/ml) and flavonoids (0.046 ± 0.014 mg/ml) were present in relatively lower concentrations. The antioxidant activity assessed using the DPPH radical scavenging assay demonstrated that the extracts possess notable free radical scavenging ability. The methanolic extract showed considerable antioxidant activity, with percentage inhibition increasing in a concentration-dependent manner.

KEYWORDS: *Pistia stratiotes*, n-hexane, Alkaloids, glycosides.

INTRODUCTION

PHYTOCHEMISTRY

Phytochemistry is the study of phytochemicals, which are chemicals derived fromplants. Phytochemistsstrive to describe the structures of the large number of secondary metabolites found in plants, the functions of these compounds inhuman and plant biology, and the biosynthesis of these compounds. Plants synthesize photochemical for many reasons, including to protect themselves against insect attacks and plant diseases. The compounds found in plants are of many kinds, but most can be grouped into four major biosynthetic classes: alkaloids, phenylpropanoids, polyketides, and terpenoids.

HISTORY OF MODERN PHYTOCHEMISTRY

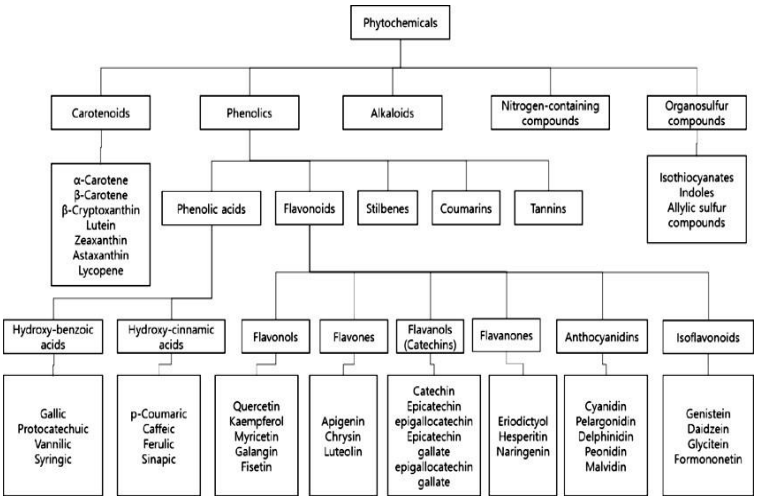
The Ethnobotanical studies of medicinal plants for the treatment of diseases have existed since antiquity. For instance, the discovery of quinine marked the first successful use of chemical compounds to treat infectious disease. This was considered as the most important medical discovery of the17th century. But in practical terms, the use of the quinine source, that is, the bark of the Cinchona (quinaquina) tree dated back as at the 16th century. However, the beginning of the isolation of plant chemical compounds marked the early stages of modern phytochemistry. This isolation not only led to the synthesis of new drugs but also to the purification of plant extracts used as medicines. It is important to note that apart from being the first to isolate an alkaloid, morphine, Friedrich Wilhelm was the first person to isolate an active ingredient associated with a medicinal plant or herb. Not long enough, his discoveries transformed pharmaceutical chemistry from a state of alchemy to an acknowledged branch of science.

PHYTOCHEMICALS

Phytochemicals are simply plant-derived chemicals. The word “phyto” comes from the Greek word plant. It is used to refer to the secondary metabolites produced by plants. As noted earlier, these metabolites are usually synthesized as a measure for self-defense against insects, pests, pathogens, herbivores, ultraviolet exposure, and environmental hazards.

Phytochemicals differ from the essential nutrients (primary metabolites) such as the carbohydrates, proteins, fats, minerals, and vitamins that are needed for the day to day maintenance of the plants. Sometimes, phytochemicals are used to refer to functional foods with antioxidant properties, nutriceuticals, phytonutrients, anti-nutrients, phytotoxins, and so forth.

CLASSIFICATION OF PHYTOCHEMICALS



MATERIAL & METHODS

Pistia stratiotes, commonly known as water lettuce, is a free-floating aquatic plant widely distributed in tropical and subtropical regions. It has long been recognized in traditional systems of medicine for its therapeutic properties, including anti-inflammatory, antimicrobial, and wound-healing effects. Despite its extensive use in folk medicine, the plant remains underexplored in terms of standardized pharmacognostic parameters and detailed phytochemical profiling. Pharmacognostic standardization plays a crucial role in ensuring the identity, purity, and quality of crude plant materials, while phytochemical characterization helps in identifying bioactive compounds responsible for its medicinal properties. Therefore, the present study on *Pistia stratiotes* focuses on establishing comprehensive pharmacognostic standards and evaluating its Phytochemical constituents, with special emphasis on correlating these findings to its pharmacological potential.

Collection of plant materials

Healthy and mature specimens of *Pistia stratiotes* (water lettuce) were collected from freshwater bodies such as ponds or slow-moving streams of Bundelkhand region. Collection was carried out during the early morning hours to ensure maximum freshness and minimal physiological stress to the plants. Only disease-free plants with well-developed rosettes were selected. The plants were carefully handpicked to avoid damage to leaves. Excess water and debris were gently removed on-site. The collected plant material was immediately transferred into clean, labeled polyethylene bags or containers filled with a small amount of native water to maintain moisture and prevent desiccation during transport. In the laboratory, the samples were thoroughly washed with running tap water to remove adhered soil particles, algae, and other contaminants. This was followed by rinsing with distilled water.

Preparation of the extract

The leaves of *Pistia stratiotes* were thoroughly rinsed with distilled water to remove adhering impurities and then shade-dried at room temperature until a constant weight was achieved. The dried leaves were subsequently ground into a fine powder using a mortar and pestle.

The leaves (2000 g) were air dried for seven days and then pulverized into powder through manual hand grinding. The powdered yield of the plant material were 1650 g (82.5%) which was divided into two parts (825 g) each macerated separately in 2.5 L of methanol and n-hexane respectively and allowed to stand for 72 hrs and then filtered. The residue was again macerated separately in 500ml of methanol and n-hexane and allowed to stand for 24 hrs and then filtered. Each extracts were concentrated to dryness at 40^0C under a reduced pressure using rotary evaporator to give a yield of 1.391g and 1.286g for methanol and n-hexane respectively. The dried extract was then transferred into universal bottles, labelled appropriately and kept in a refrigerator for further experimental analysis.

Figure: Extracts of leaves of *Pistia stratiotes.*

Determination of percentage yield

Percentage yield is an important parameter for evaluating the efficiency of the extraction process. It indicates the amount of extract obtained relative to the initial quantity of plant material used. The% yield was calculated by using formula-%yield =[(weight of dried extract)/(weight of dried plant sample)]x 100

Screening for Phytochemicals

Preliminary phytochemical screening of the methanolic and n hexane leaf extracts of *Pistia stratiotes* were carried out to identify the presence of major bioactive constituents using standard qualitative methods. The extracts were subjected to various chemical tests for the detection of different classes of phytochemicals.

Different plant metabolites and their test procedures test for alkaloids

1. **Hager's test:** to a few ml of filtrate, 2 drops picric acid was added formation of yellow precipitate shows a positive result for alkaloids.
2. **Wagner's test (iodine - potassium iodine reagent):** To about an ml of extract few drops of Wagner's reagent were added. Reddish - brown precipitate indicates presence of alkaloids.

Test for flavonoids

A) Alkaline reagent test: To 5ml of extract 2ml of NaOH was added by which solution turns yellow colour, further dilute HCI (0.1N) was added the solution becomes colourless which indicates the presence of phenol.

B) Lead acetate test: To 5 ml of extract few drops of lead acetate solution was added and mixed gently. The production of bulky white precipitate is positive for flavonoid.

Detection of glycosides: Extract was treated with dil. H) SO4, formation of red color solution indicate the presence of glycosides.

Test for carbohydrate

A) Benedict's test: About 0.5 ml of the filtrate was taken to which 0.5 ml of Benedict's reagent is added. This mixture was heated for about 2 minutes in a boiling water bath. The appearance of red precipitate indicates the presence of sugars.

B) Fehling test test: About 0.5 ml of the filtrate was taken to which 0.5 ml of each Fehling A & Fehling B solution was added. This mixture was heated for about 2 minutes in a boiling water bath. The appearance of red precipitate indicates the presence of sugars.

Test for phenol

A) FC reagent test: To 5ml of extract 2ml of Folin-Ciocalteu reagent is added. Appearance of blue green colour indicates the presence of phenol.

B) Ferric chloride test: To 5 ml of extract few drops of feeric chloride solution was added and mixed gently. The production of blueish black colour solution indicate presence of phenols.

Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Detection of saponins

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of proteins

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Quantitative estimate on of phytochemicals

The quantitative estimation of major phytochemicals present in the extract of *Pistia stratiotes* was carried out using standard spectrophotometric and gravimetric methods. These analyses provide precise information regarding the concentration of bioactive compounds present in the plant extract.

Estimation of total phenolic content

The total phenolic content was determined using the Folin–Ciocalteu method. The extract was mixed with Folin–Ciocalteu reagent followed by the addition of sodium carbonate solution. The mixture was incubated, and the absorbance was measured at 765 nm using a UV–Visible spectrophotometer. The phenolic content was expressed as mg of gallic acid equivalents (GAE) per gram of dry extract.[75]

Reagents

- Folin-ciocalteureagent
- Sodiumcarbonatesolution Gallic acid (standard)

Procedure

The total phenolic content of *Pistia stratiotes* extract was performed with folin-ciocaltaeu assay. 2 ml of sample (11 mg/ml) was mixed with 1 ml of folin ciocalteu's phenol reagent and 1 ml of (7.5 g/L) sodium carbonate solution was added and mixed thoroughly. The mixture was kept in the dark for 10 minutes at room temperature, after which the absorbance was read at 765 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the Phenolic compounds was carried out in triplicate. The TPC was expressed as 100 milligrams of Gallic acid equivalents (GAE) 100mg of dried sample.[76]

Estimation of total flavonoids content

In the present investigational uminumchloride colorimetric method is employed for the quantitative estimation of flavonoids present in *Pistia stratiotes* extract. The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols.[77]

Reagents

- 2%AICI3
- Quercetin(standard)

Procedure

Preparation of standard solution10mg quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1 ml was pippeted out and made up to 10ml with Methanol to get 100ug/ml Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 5, 10, 15, 20 and 25 ug/ml were prepared. 3 ml of each standard and test was mixed with 1 ml of 2% Aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 420 nm using UV-Visible spectrophotometer. A standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance.

Saponins Quantification

According method adopted by Obdoni *et al*., (2001),[78] a conical flask was filled with two grams (2g) of pulverized material and of 20% aqueous ethanol. The samples were boiled in a water bath at 550C for 0.4 hours with constant stirring. 2 ml of diethyl ether was added to the resulting mixture through a250 ml separating funnel, and it was violently agitated. The ether layer was discarded and the aqueous layer was collected. After that, 6 ml of n-butanol was added, followed by 1 ml of 5% aqueous sodium chloride (NaCl). The rest of the solution was warmed in a water bath. The sample was dried in the oven to a consistent weight after evaporation.

Saponins(mg/g)= Weight of residue/Weight of sample taken

Alkaloids Quantification

The technique of Chattheeranant *et al.*, was used to determine alkaloids. Onegram (1g)of crude extract and its fractions were weighed into a250 mlbeaker, and then 200 ml of 10% acetic acid in ethanol was added capped and left to stand for 4 hours. It was filtered, and the extract was concentrated to one quarter of its original volume in a water bath heated at 100°C. Drop by drop, concentrated ammonium hydroxide (NH4OH) was added to the extract until it precipitated. The precipitate was collected, rinsed with dilute ammonium hydroxide (NH4OH), and filtered when the Solution had settled. The alkaloid residue was dried in an oven, weighed, and the total alkaloids were determined

Alkaloids(mg/g)=Weight of residue/Weight of sample

DPPH free radical scavenging assay

The free radical scavenging activity (FRSA) of methanol and hexane extracts of *P. stratiotes* was determined against DPPH by measuring UV absorbance at 517nm employing a slightly modified method.[80] The concentrations range of extract(20-300 µg/ml) was prepared separately in methanol and n-hexane respectively. Ascorbic acid was used as reference standard and same concentrations were prepared as the test solution. A 2.0ml of each concentration were placed into test tubes and 0.5ml of 1ml DPPH solution in methanol (or n-hexane) was added and samples incubated for 15 minutes at room temperature. The UV/Visible absorbance was read at 517nmand the experiment was in triplicates, using 1ml DPPH solution in methanol (or or n-hexane) as the blank solution. A lowered absorbance of the reaction mixture indicates a FRSA, which was calculated using the formula:

RESULT

Results of extractive values

The percentage yield of methanolic and n-hexane extracts of leaves of Pistia stratiotes as shown in table.

Table: Extractive value of leaves of *Pistia stratiotes.*

Extract	%Yield(W/W)
Methanolic extract of Leaves of Pistia stratiotes	17%
n-hexane extract of leaves of Pistia stratiotes	10.5%

Phytochemical screening

Phytochemical analysis was performed to identify the bioactive constituents present in the leaf extracts of Pistia stratiotes. The methanolic and n- hexane extracts of the leaves showed positive results for several phytochemicals, including flavonoids, alkaloids, saponins, amino acids, tannins, and carbohydrates. The presence of multiple classes of compounds in the methanolic and n- hexane extracts indicates its high extraction efficiency, suggesting that methanol is a suitable and effective solvent for isolating phytochemical constituents from the leaves.

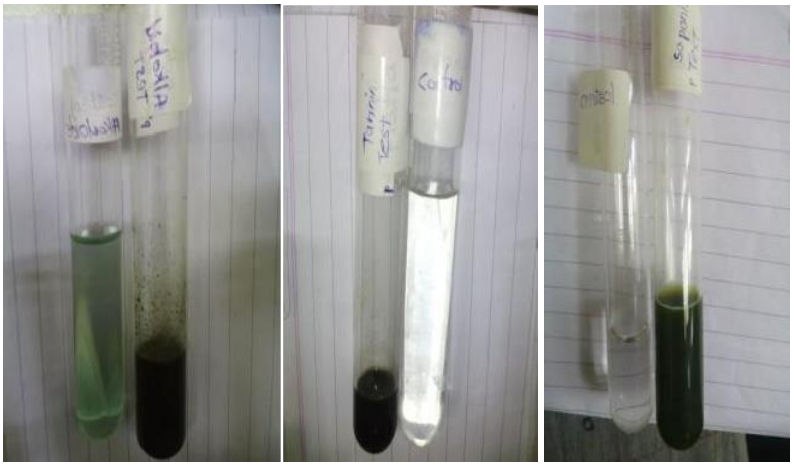
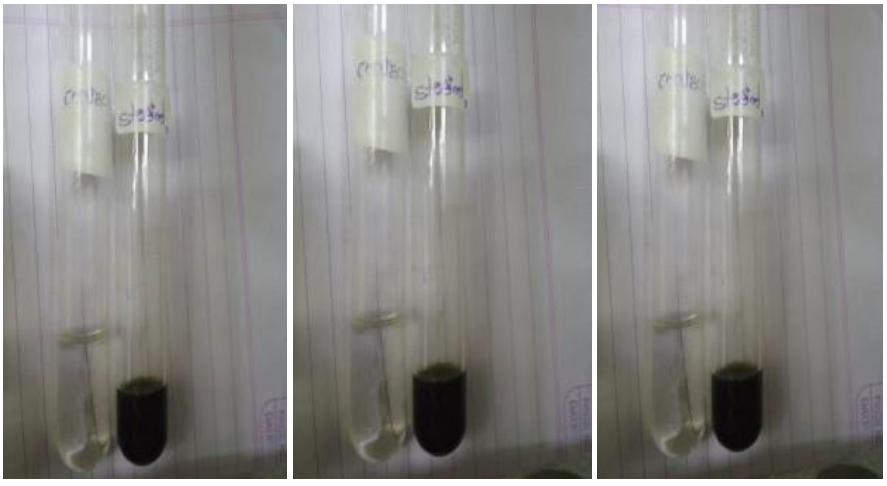


Figure: A Phytochemical tests for extract of leaves of Pistia stratiotes.



a) Steroids b) Amino acids c) Carbohydrates

Figure 7: 1b Phytochemical tests for extract of leaves of Pistia stratiotes.

Table: Different phytochemical tests and results.

S/N	Secondary metabolites	Methanolic extracts of *Pistia stratiotes*	n-hexane extracts of *Pistia stratiotes*
1	Flavonoid	+	+++
2	Tannins	+	+++
3	Alkaloid	++	+++
4	Glycosides	++	+
5	Reducingsugar	+	++
6	Anthraquinones	-	-
7	Resins	+	-
8	Saponin	++	--
9	Keller-killianitestfor Deoxy sugar	+	+
10	Phenols	+	+

Quantitative Analysis of *Pistia stratiote*s alcoholic leaf extract

The quantitative phytochemical study of alcoholic leave extract of *Pistia stratiotes* showed the highest maximum concentration of saponin (2.42± 0.56 mg/ml) was found to be the greatest, followed by Phenol (1.44± 0.055 mg/ml) and Alkaloids (0.058±0.06mg/ml) and Flavonoids(0.046±0.014mg/ml) were lowest Concentration of phytochemical. The quantitative analysis of the alcoholic leaf extract of *Pistia stratiotes* was shown in Table 7.3.

Table 7.3: Quantitative Phytochemical Constituents of *Pistra stratiote*s alcoholic leaf extract value is expressed as mean ± SEM for three determinations.

S. No	Phytochemical Constituents	*Pistiastratioles*
1	Phenol	1.44± 0.055(mg/ml)
2	Flavonoids	0.046±0.014 (mg/ml)
3	Saponins	2.42± 0.56(mg/ml)
4	Alkaloids	0.058±0.06 (mg/ml)

In-vitro antioxidant activity

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity(DPPH)

The DPPH radical scavenging assay was employed as a preliminary method to evaluate the antioxidant potential of plant extracts, based on their ability to donate protons and neutralize free radicals—an essential mechanism of antioxidant action. The free radical scavenging activity (FRSA) of methanol and hexane extracts of *P. stratiotes* was determined against DPPH by measuring UV absorbance at 517nm: Free radicals cavenging activity of *Pistia stratiotes* extracts compared with Vitamin C standard.

Conc. (µg/ml)	Mean absorbance			%Inhibiton		
	P. stratiotes (methanol extracts)	*P. stratiotes (n-hexane extracts)*	Vitamin C standard	*P. stratiotes (methanol extracts)*	*P. stratiotes (nhexane extracts)*	Vitamin C
20	0.425	0.418	0.292	31.5	32.5	52.9
40	0.406	0.394	0.281	34.5	36.5	54.7
60	0.404	0.374	0.257	34.8	39.7	58.6
80	0.403	0.344	0.238	35.0	44.5	61.6
100	0.393	0.321	0.176	36.6	48.2	71.6
120	0.391	0.315	0.155	37.0	49.2	75.0
140	0.384	0.312	0.131	38.1	49.7	78.9
160	0.369	0.307	0.125	40.5	50.5	79.8
180	0.357	0.305	0.117	42.4	50.8	81.1
200	0.351	0.291	0.087	43.4	53.1	86.0
220	0.258	0.273	0.068	58.4	60.5	89.0

240	0.246	0.245	0.053	64.6	60.5	91.5
260	0.246	0.246	0.046	64.8	60.3	92.6
280	0.247	0.247	0.043	65.1	60.2	93.1
300	0.247	0.246	0.041	65.2	60.3	93.4

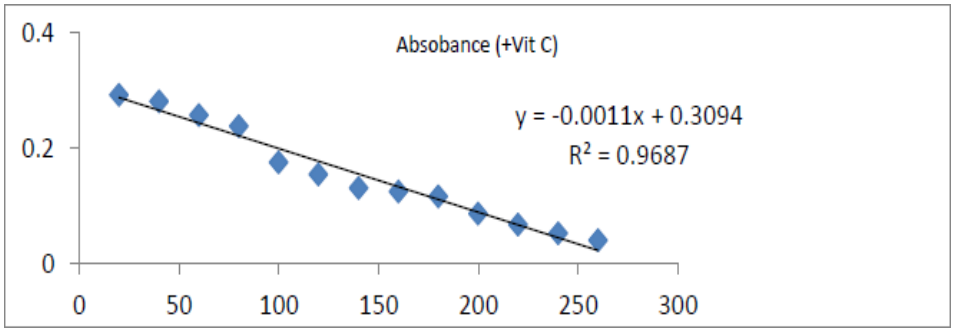


Figure: Calibration curve of absorbance versus concentration (µg/ml) for Vitamin C standards.

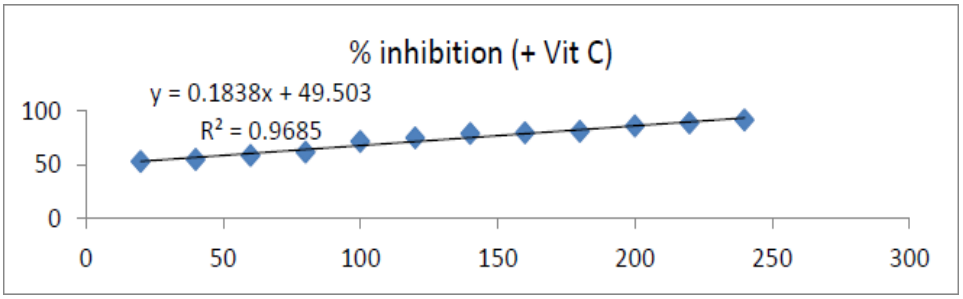


Figure: Calibration curve of % inhibition versus concentration (µg/ml) for Vitamin C standards.

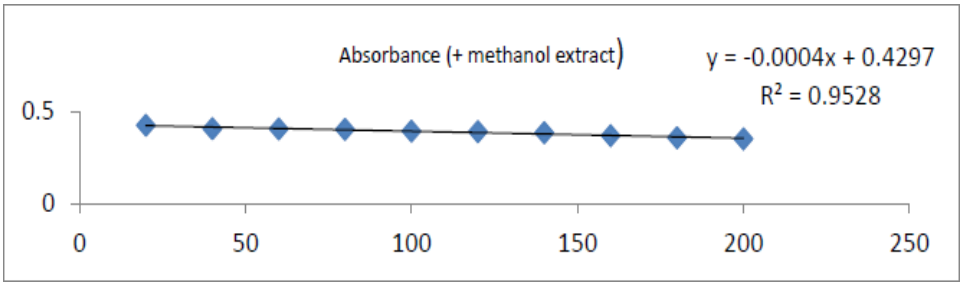


Figure: Calibration curve of absorbance versus concentration (µg/ml) for methanolic extract of *Pistia stratiotes.*

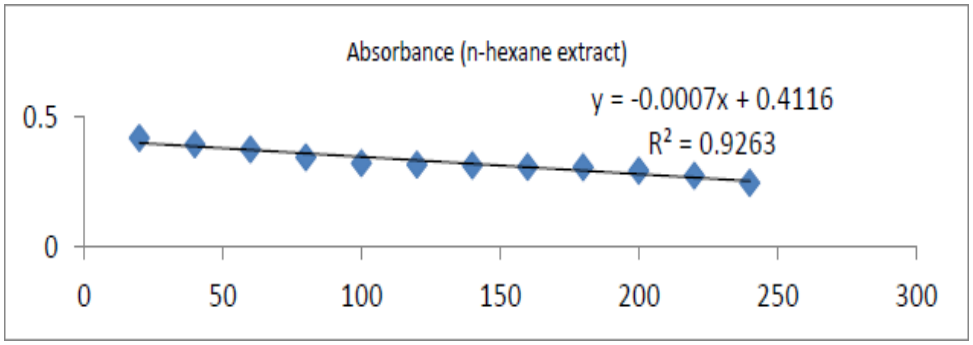


Figure: Calibration curve of absorbance versus concentration (µg/ml) for n-hexane extract of *Pistia stratiotes.*

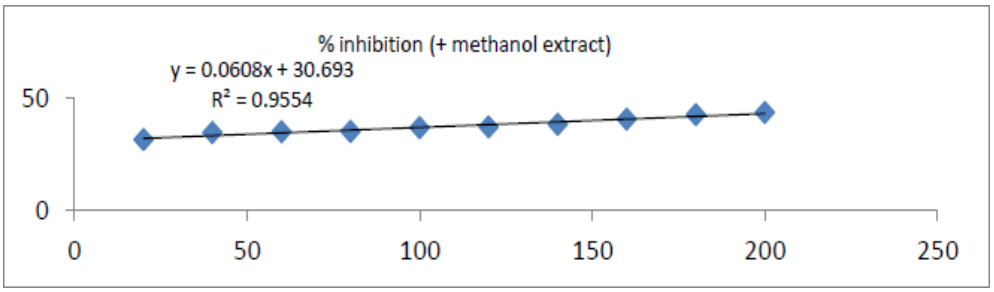


Figure: Calibration curve of % inhibition versus concentration (µg/ml) for methanol extract of *Pistia stratiotes.*

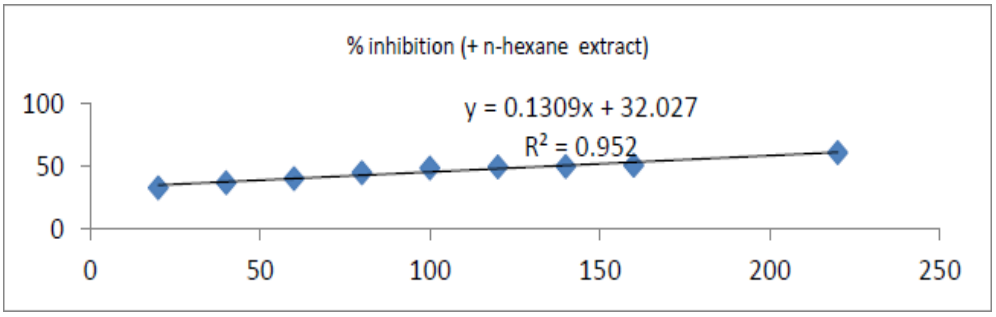


Figure: Calibration curve of % inhibition versus concentration (µg/ml) for n-hexane extract of *Pistia stratiotes.*

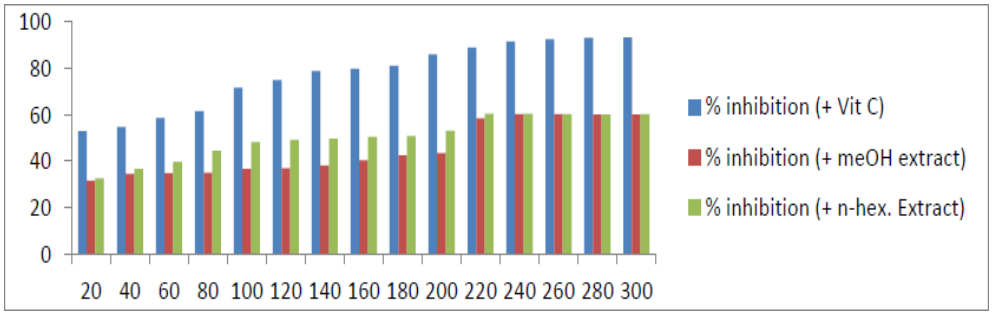


Figure: Graphical illustration of % inhibition of extracts of *P. stra-tiotes* in comparison with Vitamin C at various concentrations (µg/ml).

DISCUSSION

The present study evaluated the extractive values, phytochemical composition, quantitative constituents, and antioxidant activity of *Pistia stratiotes* leaf extracts using methanol and n-hexane as solvents. The extractive value results revealed that the methanolic extract exhibited a higher yield (17% w/w) compared to the n-hexane extract (9% w/w). This difference indicates that methanol, being a polar solvent, is more efficient in extracting a broader range of phytoconstituents from *Pistia stratiotes* leaves than the non-polar n-hexane. The higher yield also suggests the abundance of polar compounds in the plant material. Preliminary phytochemical screening confirmed the presence of several important bioactive compounds, including flavonoids, alkaloids, tannins, glycosides, phenols, and carbohydrates in both extracts. However, the methanolic extract showed comparatively better extraction of key phytochemicals such as saponins and glycosides, while n-hexane extract demonstrated stronger presence (+++) of flavonoids, tannins, and alkaloids. The absence of anthraquinones in both extracts indicates their negligible presence in the plant. Overall, the results suggest that solvent polarity plays a crucial role in determining the type and extent of phytochemical extraction. Quantitative analysis of the alcoholic (methanolic) leaf extract further supported the qualitative findings. Among the phytoconstituents, saponins were found in the highest concentration (2.42±0.56mg/ml),

followed by phenols (1.44±0.055 mg/ml). Alkaloids (0.058 ± 0.06 mg/ml) and flavonoids (0.046 ± 0.014 mg/ml) were present in relatively lower concentrations. The high content of saponins and phenolic compound significant, as these compound sare well known for their biological and pharmacological activities, particularly antioxidant potential. The antioxidant activity assessed using the DPPH radical scavenging assay demonstrated that the extracts possess notable free radical scavenging ability. The methanolic extract showed considerable antioxidant activity, with percentage inhibition increasing in a concentration-dependent manner. At higher concentrations, both methanolic and n-hexane extracts exhibited substantial activity; however, the methanolic extract showed better consistency due to its richer phytochemical profile.

CONCLUSION

The present study successfully established the pharmacognostic standardization and phytochemical characterization of *Pistia stratiotes* leaves, with a particular emphasis on their antioxidant potential. The findings clearly demonstrate that solvent selection plays crucial role in the extraction of bioactive constituents, with methanol proving to be a more efficient solvent than n-hexane, as evidenced by its higher extractive yield (17% w/w). Phytochemical screening confirmed the presence of a wide range of biologically active compounds such as flavonoids, alkaloids, tannins, saponins, glycosides, and phenolic compounds. The in-vitro antioxidant activity of *Pistia stratiotes* was evaluated using the DPPH radical scavenging assay, where the free radical scavenging activity (FRSA) of methanolic and n-hexane extracts was determined by measuring absorbance at 517 nm. The results demonstrated a clear concentration-dependent increase in percentage inhibition for both extracts, indicating their ability to effectively neutralize DPPH free radicals. Among the tested extracts, the methanolic extract exhibited comparatively better antioxidant activity, while the n-hexane extract also showed appreciable activity at higher concentrations. Overall, the study highlights that *Pistia stratiotes* is a rich source of phytochemicals with significant antioxidant activity, supporting its potentialuse in the development of natural antioxidant agents and herbal formulations. Furthermore, the pharmacognostic and phytochemical data generated in this study can serve as a valuable reference for the identification, standardization, and future research on this plant.

REFERENCES

1. David, S., & Jacoby, I., Deconstructing the history of quinine.*Trendsin Pharmacological Sciences*, 2005; *26*(11): 552–558.
2. Achan,J., Talisuna,A.O., Erhart, A., Yeka,A., Tibenderana,J. K., Baliraine,F. N., Rosenthal, P. J., & D'Alessandro, U., 2011.
3. Krishnamurti, M., & Rao, K.V., The history of phytochemistry.In *Phytochemistry: Anoverview*. Springer, 2016.
4. Hahn, M.G., Plant cell wall polysaccharides. In *Biochemistry of plants* (Vol.14).AcademicPress, 1998.
5. Ramawat, K. G., Dass, S., & Mathur, M., The chemical diversity of bioactive molecules and therapeutic potential of medicinal plants. In K. G. Ramawat (Ed.), *Herbal drugs: Ethnomedicine to modern medicine*, 2009; (pp. 7–32).
6. Walton, N. J., Mayer, M. J., & Narbad, A., Vanillin. *Phytochemistry*, 2003; *63*(5): 505–515. https://doi.org/10.1016/S0031-9422(03)00149-3
7. Dai, J., & Mumper, R. J., Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 2010; *15*(10): 7313–7352.
8. Pridham, J. B., Phenolics in plants in health and disease. *Pergamon Press*, 1960.

9. Harborne, J. B., *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Springer, 1999.
10. Schofield, P., Mbugua, D.M., &Pell, A. N., Analysis of condensed tannins: A review. *Animal Feed Science and Technology,* 2001; *91*(1–2): 21–40.
11. Elbein, A. D., Pan, Y. T., Pastuszak, I., & Carroll, D., New insights on trehalose: A multifunctional molecule. *Glycobiology*, 1999; *9*(4): 341–350.
12. Harborne, J. B., *The flavonoids: Advancesin research since 1986*. Chapman & Hall, 1991.
13. McCaskill, D., & Croteau, R., Some caveats for bioengineering terpenoid metabolism in plants. *Trends in Biotechnology,* 1998; *16*(8): 349–355.
14. Azwanida, N. N., A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal & Aromatic Plants,* 2015; *4*(3): 196. https://doi.org/10.4172/2167-0412.1000196.
15. Harborne, J. B., *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Springer, 1998.
16. Houghton, P.J.,&Raman, A., *Laboratory handbook for the fractionation of natural extracts*. Springer, 1998.
17. Kokate, C. K., Purohit, A. P., &Gokhale, S. B., *Pharmacognosy* (42nd ed.). Nirali Prakashan, 2010.
18. Pandey, A., & Tripathi, S., Concept of standardization, extraction and pre phytochemical screening strategies for herbaldrug. *Journal of Pharmacognosy and Phytochemistry,* 2014; *2*(5): 115–119.
19. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & YogaLatha, L., Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and AlternativeMedicines,* 2011; *8*(1): 1–10.
20. Trease, G.E., &Evans, W. C., *TreaseandEvanspharmacognosy* (16thed.). Saunders Elsevier, 2009.
21. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H., Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia,* 2011; *1*(1): 98–106.
22. Halliwel, B., & Gutteridge, J. M.C., *Free radicals in biology and medicine* (5thed.). Oxford University Press, 2015.
23. Warner, K., & Knowlton, S., *J. Am. Oil Chem. Soc.*, 1997; *74*: 1317.