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Bioactivity Guided Characterization of Methanolic Extract of Pistia Stratiotes Leaves in Lucknow, Uttar Pradesh

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ABSTRACT:

Background: *Pistia stratiotes*, Araceae family, traditionally used in tropical and subtropical regions for medicinal purposes is found through all tropical and subtropical freshwater streams. It is regarded as an invasive species. It is known to be rich in secondary metabolites targeted at medicinal effects.

Aim and objectives: The study aimed to authenticate, extract and perform bioactivity guided characterization of extracts. After DPPH assay for antioxidant activity, identification of phytoconstituents was intended.

Material and Methods: Phytochemical screening and antioxidant assay was performed to select most potent extract which was followed by characterization parameters like Total phenols, Total flavonoids, HPTLC and gas chromatography mass spectrometry (GC-MS) studies for identification of phytoconstituents.

Results: Phytochemical screening revealed presence of numerous phytoconstituents in methanol extract. Of the 3 solvent extracts tested, percentage yield varied from methanolic extract (4.5±0.60) ethyl acetate (3.7 ±0.34) and then in aqueous extract (5.5±0.45). Total phenolic content recorded in methanolic extract, was 240±0.03 mg gallic acid equivalent per gram while total flavonoid content was 260±0.01 mg quercetin equivalent per gram. Bioactivity guided identification of various potent constituents was thus achieved.

Discussion: The bioactivity guided characterization resulted in identification of methanolic extract as most potent as reflected in GCMS profile.

Conclusion: We conclude that methanolic extract with considerable antioxidant potential might be tested for additional pharmacological activities owing to rich presence of phytochemicals.

INTRODUCTION

Bioactivity-oriented assessment strategies assist isolation and purification of key phytoconstituents which enable efficient identification of specific phytoconstituents. It is also imperative where cost effective discoveries can be achieved as their pharmacological activity is pre-determined (Han et al., 2018 & Shi et al., 2010). Ample of work done in this direction has earned

successful findings in strategic drug discovery. In a study on methanolic leaf extract of *Searsia lancea,* fractions were screened for antibacterial properties followed by preparative thin layer chromatography. Further, phytocompounds were characterized and quantified using Gas Chromatography-Mass Spectrometry (GC-MS) (Vambe et al., 2021). Bioassay-guided fractionation from *Acacia hydaspica* led to isolation of bio-constituents which were characterized by nuclear magnetic resonance and spectroscopic techniques (Afsar et al., 2022). Das et al, performed bioactivity-guided assay of *Avicennia officinalis* which led to isolation of a bioactive compound which was analysed by different spectral techniques (Das et al., 2019).

Another prevalent topic over the last two decades is oxidative stress as it is commonly associated with several diseases including autoimmune and inflammatory diseases (Rahal et al., 2014 & Sosa et al., 2013). Oxidative stress is a signal that reflects the overwhelming production of reactive oxygen species that exceeds the antioxidant capacity within a cell. Prolonged oxidative stress can damage cellular building blocks, disrupt cellular signalling, and induce the release of inflammatory signalling molecules (Poli et al. 2004 & Hussain et al., 2016). Recent statistics have shown that approximately 85% of people use plant extracts to treat various diseases in the country based on their healthcare needs (Lu et al., 2014).

Due to inadequate health centres, shortage of medicines and low-income community, almost 70% people in developing countries mainly depend on reliable natural compounds (Yenet et al., 2023; Adebisi et al., 2022; Eden et al., 2019). Phytoconstituents like flavonoids, tannins, alkaloids have been obtained with largely insignificant toxicities and immunogenic properties (Brindha et al., 2016). One such natural resource , *P. stratiotes*, in the family of Araceae, is known for its medicinal uses (Khan et al., 2014 and Rokaya et al., 2023).

P. stratiotes, or water lettuce, is found through all tropical and subtropical freshwater streams. It is regarded as an invasive species. Literature reports claim its richness in phytochemicals belonging to chemical classes such as alkaloid, flavonoids, glycosides, saponins and tannins etc (Agarwal et al., 2022). It is known to reduce swelling, stress, and has antimicrobial effect (Netshiluvhi et al., 2016). Phenolic compounds present in varied chemical forms contribute to antioxidant action in plant foods (Asif et al., 2015). Proper extraction and isolation techniques are crucial in the success of such therapies where commonly used techniques such as maceration, Soxhlet and microwave-assisted extraction have been popularly used (Kalaskar et al., 2025). Polar solvents like ethanol, water and methanol are employed to obtain polar compounds (Górecki et al., 1998). Not much reliable information is available for *P. stratiotes* despite wide applications in medicine system. Bioactivity guided fractionation is a popular strategy for validating the pharmacological potential leading to effective management of resources. Therefore, we intend to optimize an extract for possible fractionation and isolation of phytoconstituents for establishing its medicinal attributes.

MATERIALS AND METHODS

Plant materials

P. stratiotes were gathered from BKT, Lucknow. Prof. N. K. Dubey, a curator at Banaras Hindu University, identified the species, and assigned a voucher specimen number (Ara 2023/01).

Sample preparation and extraction

The plant leaves were treated with deionised water to remove soil. It is then, coarsely powdered followed by extraction with petroleum ether, methanol, ethyl acetate and aqueous solvents by the using Soxhlet apparatus until the content of siphon arm turned colourless. The resultant residue was stored at 40^{o} C for subsequent analysis (Meena et al., 2020).

Pharmacognostic studies

The macroscopic and microscopic characterization of fresh leaves of *P. stratiotes* were observed through standard methods as per WHO (Sarfraz et al., 2024).

Instruments

UV-Visible Double Beam Spectrophotometer (PC-Based Double Beam UV-VIS Spectrophotometer 2201), Mettler Toledo Fp 20 Bio Kit pH Meter, (GC, Agilent 8890 GC-MS system, Germany), (MS, 5977B GC(MSD), USA), refrigerator (Lec 1602, England), vortex mixer(Abron, India), magnetic stirrer (BiBy sterline LTD, staford shire ST 15 OSA, UK), grinder (IKA-WRKE, GMBH & CO.KG D-79219 staufen, Germany) and Whatman filter paper (No. 42) Weighing balance- Toledo Balance XPR204S Mettler.

Extraction of Plant Material

The leaves were grinded and crushed to a fine powder, after washing and air drying at room temperature. Thereafter, the powdered plant (1000g), was extracted by solvents in successive manner with increasing polarity. The solvents selected were petroleum ether, ethylacetate, methanol and water, and after each cycle, extracts were concentrated to dryness using rotary evaporator. The extract were properly stored in bottles and kept in a desiccator with anhydrous sodium sulphate. Subsequent storage was made in stoppered bottles within a refrigerator at 20°C (Sugumar et al, 2025).

Qualitative phytochemical screening

Phytoconstituents were determined qualitatively using different chemical methods and thin-layer chromatographic analysis (Sureshkumar et al., 2021).

The methanolic, chloroform, ethyl acetate and aqueous extracts of *P. stratiotes* were qualitatively evaluated to detect the presence of different phytochemicals like alkaloids, glycosides, flavonoids, tannins, saponins and sterols by the standard procedure (Tyagi et al., 2017).

Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the different plant extracts were compared by adapting the procedure reported by Shah et al. (Shah et al., 2015). Each extract was dissolved in an appropriate volume of methanol to give final concentrations ranging from 10 to 250 mg/mL. Standard ascorbic acid solutions were also prepared in methanol to give the same concentration range.

Two millilitre of each solution was transferred to a test tube and mixed with 4 mL of 0.3 mmol/L DPPH. A control solution was prepared by adding methanol (2 mL) to 0.3 mmol/L DPPH (4 mL). The experiment was run in triplicate. The solutions were mixed well and left in the dark for 30 min. After 30 min the solutions were analysed on a UV–vis spectrophotometer at 517 nm. The percentage of antioxidant potential was calculated using the formula:

% Inhibition = Ac− As/ Ac × 100

where, Ac is absorbance of the control solution, As is absorbance of the sample or standard.

Thin-layer chromatography

Thin layer chromatography of various extracts of *P. stratiotes* leaves was performed using three different solvent systems, Chloroform: methanol (9:1), Benzene: acetone (2:1), and Chloroform: ethylamine benzene to detect flavone, anthocyanidin flavanols, and phenolic acids respectively. Temperature was maintained at 110 °C until spots were visible. Every spot with a specific Rf on the TLC plate indicates the presence of a specific compound, as per the method described by (Kabore et al., 2023).

HPTLC (HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY)

Sample preparation

Extract residue was re-dissolved in 1 ml of chromatographic grade methanol, which was used for sample application on precoated silica gel 60F 254 HPTLC plates of 0.2 mm thickness. **Developing solvent system,**

The mobile phase composition was optimized by testing various solvent systems and a satisfactory resolution was obtained in the solvent, Toluene: ethyl acetate: formic acid (5:4:0.1).

Application of sample

Samples were applied to the precoated silica gel 60F 254 HPTLC plates of 20x20 cm thickness using Linomat 5 applicator attached to CAMAG HPTLC system, which was equipped with WINCATS software.

Development of chromatogram

After the application of sample, the plates were eluted in pre-saturated twin trough glass chamber 10x 10 cm at room temperature with the mobile phase Toluene: ethyl acetate: formic acid (5: 4: 0.1).

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Detection of spots

The air-dried plates were viewed in UV radiation to mid-day light. The spots were visualized under CAMAG UV cabinet 254 and 366 nm. Then the chromatograms were scanned in the densitometer using CAMAG TLC scanner III. The finger print data and the Rf values were recorded by WINSCATS software. **(**Gope et al., 2024).

Physico-chemical analysis

Physico-chemical parameters like ash values (total ash, acid insoluble ash, water soluble ash), moisture content, were evaluated on the shade dried powdered crude drug **(**Bhardwaj et al., 2023)

Total Phenolic content

A modified method for the determination of total phenolic content was carried out using modification of the method cited by Sahu and Saxena (Sahu et al., 2013). Gallic acid standard solutions were prepared in methanol to give the following final concentrations; 20, 40, 60, 80, and 100 mg/mL. Each plant extract was dissolved in methanol to give a final concentration of 1.0 mg/mL and 0.5 mL of each sample and standards were introduced into different test tubes and mixed with 2.5 mL of a 10-fold dilute Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate were added. The test tubes were covered with para lm and allowed to stand for 30 min at room temperature before the absorbance was read at 760 nm. The results of the total phenolics were expressed as mg of gallic acid equivalents (GAE) per gram of sample and calculated by the formula (Kumar et al., 2025**)**:

$$TPC = (C \times V)/M$$

where, TPC is total phenolic content (mg/g plant extract in GAE), C is concentration of gallic acid established from the calibration curve (mg/mL), V is volume of the extract (mL), M is mass of the extract of the plant (g).

Total flavonoid content

Total flavonoid content was estimated using standard methods with minor modifications (Matic et al., 2017 & da Silva et al., 2015) using quercetin as a standard. The standard solutions with the following final concentrations were prepared; 50, 100, 150, 200 and 250 mg/mL. One milliliter of each standard solution and extract solutions was taken into 10 mL volumetric ask, containing 4 mL of deionised water.

Then 0.3 mL of 10% $AlCl_3$ was added to the mixture. At the 6^{th} min, 2 mL of NaOH (1 mol/L) was added and volume made up to 10 mL with distilled water. The absorbance was read at 510 nm using Beckman Coulter DU 650i UV–vis spectrophotometer. The results of the total flavonoids were expressed as quercetin equivalents (QE).

GCMS ANALYSIS OF METHANOLIC EXTRACT *P. STRATIOTES*

The compounds in methanol extract of *P. stratiotes* leaves were identified based on gas chromatogram coupled with mass spectrometer (GC-MS). The GC-MS of Agilent Technologies 8890 GC system equipped with an Agilent Technologies GC-MS capillary column HP-5 MS measured with Column Elite-1 fused silica capillary column (30 m x 0.25 mm 1D x 25 µL) contained 100% dimethyl polysiloxane, 70 eV ionization voltage was for GC-MS operation. The carrier gas was helium gas (99.99%) with flow rate of 1 mL/min and 2 µL an injection volume was employed at an injector and ion source temperature of 250°C and 280°C, respectively. The identified compounds were based on the comparisons of their mass spectra with NIST library.

ETHICAL STATEMENT: This study is an in vitro characterization of herbal extract and did not involve human participants or animal subjects; therefore, ethical approval was not required.

STATISTICAL ANALYSIS: All experimental analyses were performed in triplicate, and results are expressed as mean± Standard Deviation (SD). Descriptive statistical methods were applied to evaluate antioxidant potential, organoleptic, physicochemical, and HPTLC data to ensure consistency, reproducibility, and reliability of the analytical parameters.

RESULTS

Morphological studies

The morphological features observed: leaves were light green with green flowers, present in axils. Bitter taste and odourless.

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Microscopical studies

Fig 1. shows the transverse section of petiole where the outer epidermis with multicellular trichomes is followed by multiple layer of polygonal cell. Vascular bundles were visible in ground tissue. Acicular calcium oxalate crystals are observed inside vessels.

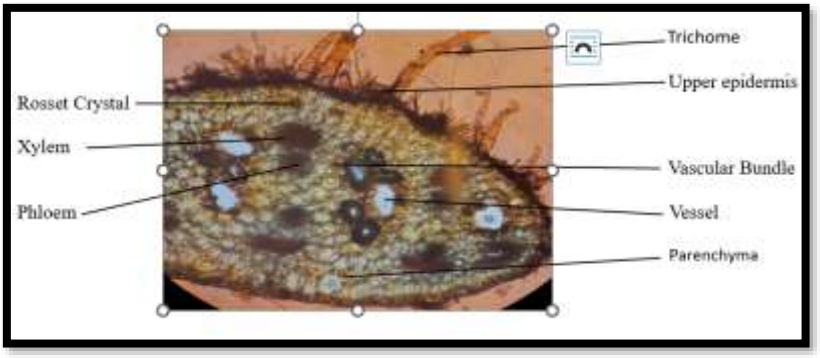


Fig. 1. Transverse section of leaf of *Pistia stratiotes*

Extractive of plant material

The extractive values were determined with petroleum ether, methanol, ethyl acetate and aqueous extracts by successive solvent extraction, which was found to be to be 1.5±0.45, 4.5±0.60, 4.0 ±0.34 and 3.5±0.45w/w, respectively. Here, the extractive values of methanolic extract and ethyl acetate extracts, were quantitatively higher than that of aqueous and Pet ether. This reflected the rich presence of polar compounds in the former two extracts.

Table 1. Extractive values of different extracts of *P. stratiotes*

Parts used	Extract	Colour	Consistency	Yield (% w/w)
Leaves	Petroleum ether	Pale green	Non sticky	1.5±0.45
	Methanol	Dark green	Sticky	4.5±0.60
	Ethylacetate	Greenish brown	Sticky	4.0 ±0.34
	Aqueous	Light brown	Non sticky	3.5±0.45

The percentage extractive values varied with different extracts where soxhlation method was selected. The enhanced extractive yields with soxhlation method were similar to reports by Mokaizh et al where they obtained 20 phenolic constituents with excellent antioxidant potential from the leaf extract of *P. stratiotes* through Soxhlet method. Therefore, soxhlation method was selected for further studies (Mokaizh et al., 2024).

Table 2: Phytochemical screening

Phytoconstituents Analyzed	Chemical Test	*Pistia stratiotes* (Extracts)			
		Pet. Ether	Ethyl-acetate	Methanolic	Aqueous
Alkaloids	Mayer's test	-	+	+	+
	Wagner's test	-	-	+	-
	Dragendorff's test	-	+	+	+
	Hager's test	-	-	+	+
Glycosides	Baljet's test	+	+	+	-
	Keller kiliani test	-	+	+	+
Flavonoids	Shinoda test	-	+	+	+

	Alkaline reagent test	-	+	+	+
Tannins	Gelatin test	-	+	+	-
	Ferric chloride test	+	+	+	+
Saponins	Foam test	-	-	+	+
Sterols	Salkowski test	-	+	+	+

The results of the phytochemical screening were also in agreement with the previous findings where all the extracts except petroleum ether, were found to be rich in polar compounds like flavonoids and tannins. The methanolic extract tested positive in all tests for all polar compounds. Similar results were obtained in *Moringa oleifera* Lam, where methanolic extract in particular had higher levels of phenolics and flavonoids. (Kristiningrum et al., 2018).

Antioxidant activity of *Pistia stratiotes*

In recent times, over exploitation of synthetic antioxidants such as BHA and BHT, which have adverse effects, necessitates search for natural antioxidants, especially plant sources (Maji et al., 2023). In multiple works, the antioxidant activity has been attributed to polyphenolics specifically flavonoids and tannins (Magiera et al., 2022 & Allagui et al., 2022). Above observation suggests the pressing need for discovery of novel antioxidant from plant resources imperative for human health. An antioxidant with low potency exhibits higher DPPH values and vice versa (Gancar et al., 2020). Unachukwu et al., realized the DPPH values of 36.07 µg/mL and 23.26 µg/mL, for *P. stratiotes* against standard, ascorbic acid **(**Unachukwu et al., 2010). Saito et al. obtained the DPPH values ranging from 8.33 to 10.10 µg/mL where they ascertained that differential method of extraction might be responsible for the variations. In yet another study, Manian and coworkers et al, found IC_{50} of *P. stratiotes* extract as 19.50 µg/mL (Saito et al., 2004). In a study by Jha et al, methanolic extract of *Pistia stratiotes* exhibited concentration dependent increase in antioxidant activity where percentage inhibition was (17.24– 79.3 %) on DPPH against reference ascorbic acid (22.7–83.4 %). (Jha et al., 2012). Zeb et al, stated in his studies that polyphenols in herbs provide hydrogen to free radical substrate for prevention of free radical formation (Zeb et al., 2020). It was also reported, that flavonoids prevent free radical production through chelation (Lee et al., 2015). Our results seem to agree with the similar findings about our plants under investigation. Some other studies by Hwang et al, reported that methanol is best for polyphenol extraction (Hwang et al., 2014; Daud et al., 2017). Previous reports about other polar extracts of *Pistia* also showed significant levels of polyphenolic and flavonoid contents (Lezoul et al., 2020; Devi et al., 2024).

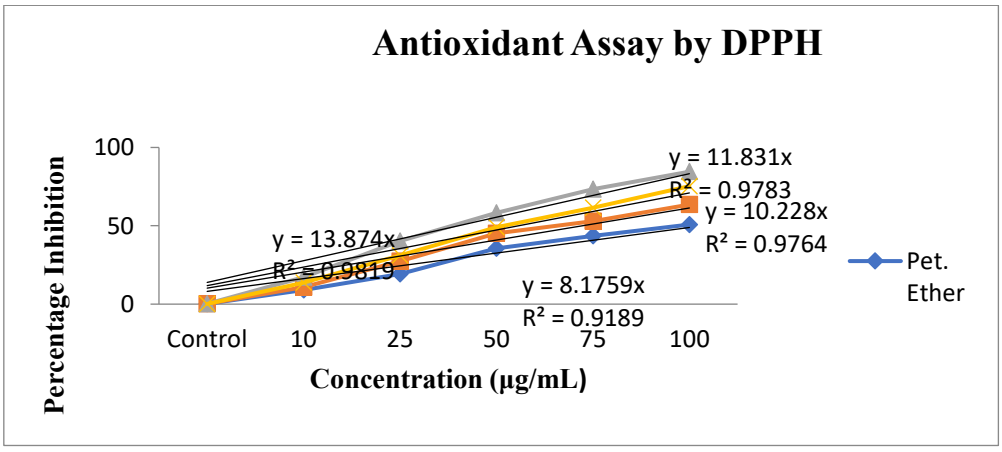


Fig. 3. Antioxidant activity of different extracts (Pet ether, Methanol, Aqueous and ethylacetate)

The methanolic extract showed promising antioxidant potential when compared to standard ascorbic acid by the DPPH scavenging assay method. The IC_{50} value was found to be 44.50 µg/mL, 58.64 µg/mL, 70.02 µg/mL for methanolic extract, aqueous extract and Ethyl acetate extracts, respectively. It signifies that methanolic extract of the plant at higherconcentration captured more free radicals formed by DPPH resulting in a decrease in absorbance.

The IC_{50} values of all the extracts was determined where the methanolic extract was most potent extract with lowest values. Therefore, characterization studies were carried out with further with Methanolic extract.

High Performance thin layer chromatography of methanolic extract of *Pistia stratiotes*

High Performance thin layer chromatography was done for the determination of phytochemicals of the methanolic extract which revealed the presence of several bio actives represented through chromatograms, at wavelengths 254 nm and 366 nm. The solvent system optimized for the estimation was Toluene: Ethyl acetate: glacial acetic acid (8:2:0.1) (Fig 4.). This was supported by optimization of solvent system as well as previous literature where flavonoids like quercetin were well separated with Mobile phase: Toluene: Ethylacetate: Glacialacetic acid: Methanol giving sharp peaks. (Das et al., 2024)



(a) 254 nm (b) 366 nm

Fig 4. HPTLC of methanolic extract of *Pistia stratiotes* was performed at 254nm and 366nm with the optimise**d solvent system as Toluene: Ethyl acetate: glacial acetic acid (8:2:0.1).** Similar findings were reported with this optimized solvent system previously, in a study by Bidkar et al. where flavonoids were confirmed. (Bidkar et al., 2021). Lakshita et al, observed results in TLC profile of O. vulgare leaves extract in Toluene-ethyl acetate-Glacial acetic acid (3:4:0.5) v/v/v, where methanol extract showed five (0.19, 0.22, 0.26, 0.53, and 0.90) spot under UV light 254 nm **(**Lakshita et al., 2025).

Physicochemical Evaluation

The results of physiochemical evaluation were as under given in the Table 3.

Table 3. Physicochemical evaluation

Parameters	Value (% w/w)
Ash Value	
Total Ash	8.63
Acid insoluble	4.20
Water soluble	2.92
Moisture Content	
	8.55

Estimation of Total Phenolic and Flavonoid Content

Plant extracts facilitate antioxidant action through their phenolic's hydroxyl groups. The solubility of extracts in polar solvents is due to hydroxyl group in phenolics (Shi et al., 2022). In our results, total phenolic content in *P. stratiotes* leaves was found to be 240±0.03 mg GAE/g of extract's dry weight which is much higher than studies of Sudirman S, et al (Sudirman et al., 2024). Additionally, flavonoid content in *P. stratiotes* methanol's leaf extract was measured using the aluminium chloride colorimetric method. Secondary metabolites called flavonoids possess antioxidant properties, and the amount and location of free OH groups determine the strength of the activity (Chandra et al., 2014**)**. The results, were expressed as quercetin equivalents (QE) per gram dry weight of the extract (Table). The total flavonoid content in the methanol extract was found to be 260 ± 0.01mg QE/g of extract's dry weight.

In support of our results, Orak HH et al. examined the total flavonoid content in the seeds, leaves, peels, and fruit pulps of *A. muricata* using aluminium chloride colorimetric method. The methanol (leaves) extract had the highest amount of total flavonoids (81.32 mg QE/g). It is understood that results of all studies can vary depending on a number of factors, including geography, environment, sample preparation technique, etc. (Orak et al., 2019).

GC-MS analysis

GC-MS analysis is a breakthrough in the analysis of phytoconstituents and structure elucidation of these compounds as they have high sensitivity in detection. GC separates mixture in to individual components and the MS detects components or molecules on the basis of their charged ion and mass to charge ratio.

In this study, the GC-MS spectrum analysis reveals the identification of twenty three compounds shown on the chromatogram depicted in Fig.5, which corresponds to the methanolic extract. Table.4 presents relative area percentages of the individual peaks of compounds. The quantified percentages for all compounds determined are as under:

The GC-MS analysis of the methanolic extract of *P. stratiotes* revealed the abundance of Butyl 9,12,15-Octadecatrienoate (5.68%), 13-Hexyloxacyclotridec-10-En-2-One (4.40%), 9-Octadecenoic Acid (Z)-, Oxiranylmethyl Ester (3.14%) while lowest abundance of Octadecanoic Acid, 2,3-Dihydroxypropyl Ester (0.09%). All 23 phytomolecules were identified and their characteristics have been mentioned along with pharmacological activities obtained through previous reports. Most of the identified compounds have been reported to have antioxidant potential.

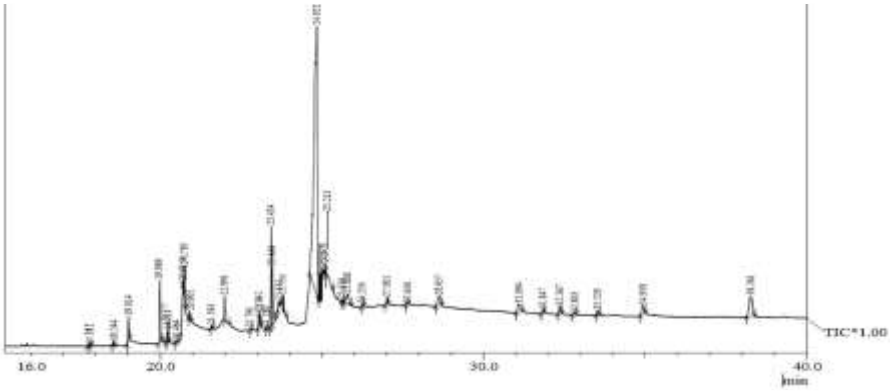


Fig 5. GC-MS chromatogram of methanol extract of *Pistia stratiotes*

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Table 4. Compounds identified in the GCMS studies

S. No.	Name of the Compound	Molecular Formula	Molecular weight	Area %	Retention Time	Medicinal use	References
1.	7-Tetradecyne	$C_{14}H_{26}$	194.36 g/mol	0.09	17.767	Antimicrobial activity	Holmberg, et al. (2007)
2.	Hexadecanoic Acid	$C_{16}H_{32}O_2$	256.42 g/mol	1.47	19.014	Anti-oxidant, Hypocholesterolaemia, antipsychotic.	Mizushina et al., (1996)
3.	13-Hexyloxacyclotridec-10-En-2-One	$C_{18}H_{32}O_2$	280.4 g/mol	4.40	19.969	Antitumor potential, antimicrobial activity	Malayaman et al. (2019)

4.	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester	$C_{19}H_{34}O_2$	294.4721 g/mol	0.91	20.177	Anticancer activity	Malayaman et al. (2019)
5.	9-Octadecenoic Acid (Z)-, Methyl Ester	$C_{19}H_{36}O_2$	296.49 g/mol	0.53	20.232	Anti-inflammatory	Jiang et al. (2015)
6.	10(E),12(Z)-Conjugated Linoleic Acid	$C_{18}H_{32}O_2$	280.4 g/mol	1.21	20.689	Inflammation insulin resistance, and dyslipidaemia	Carrasco,et al.(2020)
7.	9-Octadecenoic Acid, (E)-	$C_{18}H_{34}O_2$	282.5 g/mol	1.28	20.730	Emollient, Pulmonary oedema	Uchegbu et al. (2015)
8.	Glycidyl Palmitate	$C_{19}H_{36}O_3$	312.5 g/mol	1.82	21.991	Lipid modifications and interactions within biological systems	Taylor et al. (2022)
9.	9,12-Octadecadienoic Acid (Z,Z)-, 2,3-Dihydroxypropyl Ester	$C_{18}H_{32}O_2$	280.4 g/mol	0.54	23.042	Antioxidant	Dalei et al. (2022)
10.	Oleic Anhydride	$C_{36}H_{66}O_3$	546.9 g/mol	0.14	23.076	Anti-inflammatory, antioxidant	Peana, et al. (2002)
11.	Octadecanoic Acid, 2,3-Dihydroxypropyl Ester	$C_{21}H_{42}O_4$	358.56 g/mol	0.09	23.280	Antioxidant	Majumder et al. (2020)
12.	Butyl 9,12,15-Octadecatrienoate	$C_{22}H_{38}O_2$	334.5 g/mol	5.68	23.434	Antiplasmodial	Dalei et al. (2022)
13.	9-Octadecenoic Acid (Z)-, Oxiranylmethyl Ester	$C_{21}H_{38}O_3$	338.5 g/mol	3.14	23.466	Antioxidant	Niki et al. (1985)
14.	Myristic Acid Glycidyl Ester	$C_{14}H_{28}O_2$	228.37 g/mol	0.39	23.662	antimicrobial	Brandsch J, et al. (2008)

15.	Methyl Commate B	$C_{31}H_{50}O_3$	470.73 g/mol	5.94	24.832	anti-inflammatory	Majumder et al. (2020)
16.	E,Z-1,3,12-Nonadecatriene	$C_{19}H_{34}O_2$	294.5 g/mol	6.55	25.213	COVID-19	Majumder et al. (2020)
17.	Linoleyl Myristate	$C_{32}H_{60}O_2$	476.8 g/mol	0.56	27.001	Antimicrobial	Tassakka, et al. (2021)
18.	E,E,Z-1,3,12-Nonadecatriene-5,14-Diol	$C_{19}H_{34}O_2$	294.5 g/mol	0.41	31.847	COVID-19	Niki et al. (1985)
19.	6a,14a-Methanopicene, Perhydro-1,2,4a,6b,9,9	$C_{30}H_{50}O$	426.7 g/mol	0.85	32.367	Antimicrobial	Tassakka, et al. (2021)
20.	3a-Methoxy-9b-Lanosta-7,24-Dien-26,23-Olide	$C_{31}H_{48}O_3$	468.7 g/mol	0.18	32.803	Antioxidants	Kumar et al. (2023)
21.	Tris(2,4-Di-Tert-Butylphenyl) Phosphate	$C_{42}H_{63}O_4P$	662.9 g/mol	0.45	33.529	Antioxidant	Niki et al. (1985)
22.	Linoleyl Palmitate	$C_{34}H_{64}O_2$	504.9 g/mol	1.23	34.938	Anti-inflammatory	Peana, et al. (2002)
23.	Phytyl Linoleate	$C_{18}H_{31}O_2$	470.73 g/mol	3.76	38.261	Antioxidant	Dalei, et al. (2022)

DISCUSSION

Over the time, Researchers' interest in identifying and analysing possible secondary metabolites from therapeutic plants has been on the rise. After proper identification and structure elucidation only, their therapeutics can be confirmed. To identify bioactive molecules in this setting, it is crucial to investigate and evaluate the botanical components of therapeutic plants. Comprehending the molecular role of these bio actives could facilitate research into compound mechanisms. Selection of most potent extracts is, however, imperative for further characterization and possible isolation. It is imperative to understand that any variation in environmental factors is associated with changes in secondary metabolites as well. Subsequently, such variations in antioxidant activity and GC–MS profiles are reported across different studies. Therefore, standardization of relevant climatic and geographical location is essential to ensure reproducibility and consistency in phytochemical investigations related to *Pistia stratiotes*.

Methanol has been widely used for bioactive extraction from the plant. Methanol is also more efficient in the extraction of polyphenols especially the low molecular weight (MW) polyphenols (Do et al., 2014). The studies show that polar solvents are preferable as they provide a higher recovery yield of phytocompounds than non-polar solvents (Nawaz et al., 2020 & Mamoona et al., 2025). The higher extraction yield in our results also prompted the use of methanol extract for characterization and identification of specific phytoconstituents.

In Previous reports also, it has been ascertained that methanol is the best solvent for the extraction of leaves of *Acalypha wilkesiana* and *Atriplex halimus* where yields were about 14.67% and 24.00%, respectively (Sigidi et al., 2016). *Pistia* stratiotes has been explored for antioxidant potential previously also but we report studies from Uttar Pradesh where the values of IC_{50} by DPPH Assay seems to contradict on the basis of either geographical location or the methods used. The current investigation additionally demonstrated that the bulk of the phytochemicals derived from GC-MS chromatograms are fatty acids, followed by hydrocarbons, phenols etc. Phytyl linoleate (3.76%) and Linoleyl myristate (0.56%): These fatty acid esters exhibit antioxidant activity by acting as lipid peroxyl radical scavengers. Linoleate esters, in particular, are involved in stabilizing membranes and inhibiting lipid peroxidation (Jahan et al., 2022). Hexadecanoic acid, methyl ester (1.47%) and Myristic acid glycidyl ester (0.39%): These saturated fatty acid esters have been reported to possess mild antioxidant activity, often working synergistically with polyunsaturated compounds. Although individually their scavenging ability is limited, they contribute to the extract's lipophilic antioxidant properties. 9,12-Octadecatrienoic acid (ZZ), methyl ester (0.91%): Also known as methyl linolenate, this is an omega-3 fatty acid ester with strong radical scavenging potential (Siddiqui et al., 2022). The presence of multiple double bonds allows for electron delocalization, making it an effective antioxidant. Urs-12-en-28-al (1.69%) (a derivative of ursolic acid): A triterpenoid known for its potent antioxidant and anti-inflammatory effects (Nguyen et al., 2022). It contributes to antioxidant capacity by scavenging radicals and modulating oxidative stress-related pathways. Methyl commate B: Though found in minor quantities, these compounds belong to classes (triterpenoids and fatty acid derivatives) that have documented antioxidant properties, often contributing to the stabilization of cellular membranes and inhibition of ROS generation.

Conclusions

Consequently, achieving efficient separation is significantly challenging. To address the complexities affecting the accurate estimation of the abundance of bioactive compounds and characterization of solvents, future studies should explore the development of more sophisticated separation and purification methodologies to improve the isolation of these valuable constituents.

The antioxidant activity observed can be attributed to the synergistic effect of several identified compounds, each contributing uniquely based on their chemical structure and functional groups: Flavonoids are the various plant secondary metabolites like flavanols, flavones, and derivatives of tannins found excessively in plants with antioxidant potentials. The presence of free OH groups, especially 3-OH group is mainly responsible for the antioxidant activity of these flavonoids. Plant flavonoids can be used as potential antioxidant *in vitro* as well as *in vivo* (Kushwaha et al., 2021**)**. The research supports the use of *Pistia stratiotes* as a promising source of bioactive phytochemicals with significant antioxidant potential. GCMS findings unveiled multiple metabolites reinforcing the significant prospects for developing standardized nutraceuticals, phytopharmaceuticals, and natural antioxidant formulations for prevention of oxidative stress-associated disorders. However, Future studies involving isolation of active constituents, comprehensive safety evaluation and animal studies are required for translation products.

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ABBREVIATIONS

DPPH: 1,1-Diphenyl-2-picrylhydrazyl; GC-MS: Gas chromatography mass spectrometry; HPTLC: High performance thin layer chromatography; BHA: Butylated hydroxyanisole; BHT: Butylated hydroxytoluene; GAE: Gallic acid equivalents; QE: Quercetin equivalents; Rf: Retention factor; UV: Ultraviolet; ROS: Reactive oxygen species; MW: Molecular weight

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Summary: The study focussed on *Pistia stratiotes*, from Araceae family, traditionally used in tropical and subtropical regions for medicinal purposes. The objective of research was to authenticate the plant, prepare extracts, and perform bioactivity guided fractionation of the most potent extract. A thorough phytochemical screening, HPTLC and GC-MS analysis was undertaken. Among methanolic, ethyl acetate, and aqueous extracts, the methanolic extract showed the highest yield and strongest antioxidant activity. It also contained higher levels of phenolics and flavonoids, indicating rich phytochemical content. GC-MS characterization confirmed the presence of multiple bioactive constituents. The study concludes that the methanolic extract of *Pistia stratiotes* possesses significant antioxidant potential and may serve as a promising source for further pharmacological investigations.

REFERENCES

[1] Adebisi, Y. A., Nwogu, I. B., Alaran, A. J., Badmos, A. O., Bamgboye, A. O., Rufai, B. O., Okonji, O. C., Malik, M. O., Teibo, J. O., Abdalla, S. F., & Lucero-Prisno III, D. E. (2022). Revisiting the issue of access to medicines in Africa: Challenges and recommendations. *Public Health Challenges*, 1(2),e9.

[2] Afsar, B., & Afsar, R. E. (2023). Hypertension and cellular senescence. *Biogerontology*, *24*(4), 457-478.

[3] Agarwal, M. (2022). Phytochemical analysis and bioactive characters of aquatic weeds *Eichhornia crassipes* (Mart.) Solms and *Pistia stratiotes* L.: A review. In *Bioactives and Pharmacology of Medicinal Plants*, 313–327.

[4] Alamgir, A. N. M. (2017). *Therapeutic use of medicinal plants and their extracts: volume 1* (Vol. 73). Cham: Springer.

[5] Allagui, I., Horchani, M., Zammel, N., Jalouli, M., Elfeki, A., Kallel, C., & Hcini, K. (2022). Phytochemical characterization, antioxidant and anti-inflammatory effects of *Cleome arabica* L. fruits extract against formalin induced chronic inflammation in female Wistar rat: Biochemical, histological, and in silico studies. *Molecules*, 28(1), 26.

[6] Asif, M. (2015). Chemistry and antioxidant activity of plants containing some phenolic compounds. *Chemistry International*, 1(1), 35–52.

[7] Bhardwaj, A. K., Kashyap, K., Hait, M., Bera, S. K., & Dewangan, H. (2023). Physicochemical characterization of rhizome of *Curcuma caesia* Roxb. *ES Food & Agroforestry*, 11(7), 813.

[8] Bidkar, A., Sable, V., & Kirtane, S. (2021). Extraction, characterization and pharmacological activity and evaluation of various extracts of *Calotropis procera* leaves. *International Journal of Health Sciences*, 5(S2), 1182–1204.

[9] Brandsch, J., & Piringer, O. (2008). 2.1 Classification, Manufacture, and Processing Aids A common feature of all plastic materials is their backbone made of polymers that means natural or synthetic macromolecules composed of thousands of atoms and. *Plastic Packaging: Interactions with Food and Pharmaceuticals*, 15.

[10] Brindha, P. (2016). Role of phytochemicals as immunomodulatory agents: A review. *International Journal of Green Pharmacy*, 10(1).

[11] Meena, D. K., Sahoo, A. K., Chowdhury, H., Swain, H. S., Sahu, N. P., Behera, B. K., ... & Das, B. K. (2020). Effects of extraction methods and solvent systems on extract yield, proximate composition and mineral profiling of Terminalia arjuna (Arjuna) dry powders and solvent extracts. *J. Innovat. Pharmaceut. Biol. Sci*, *7*(1), 22-31.

[12] Carrasco, F., Pagès, P., Gámez-Pérez, J., Santana, O. O., & Maspoch, M. L. (2010). Processing of poly (lactic acid): Characterization of chemical structure, thermal stability and mechanical properties. *Polymer Degradation and stability*, *95*(2), 116-125.

[13] Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., Elsohly, M. A., & Khan, I. A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. *Evidence-Based Complementary and Alternative Medicine*, 2014, 253875.

[14] Poli, G., Leonarduzzi, G., Biasi, F., & Chiarpotto, E. (2004). Oxidative stress and cell signalling. *Current medicinal chemistry*, *11*(9), 1163-1182.

[15] Cheng, W. W., Liu, G. Q., Wang, L. Q., & Liu, Z. S. (2017). Glycidyl fatty acid esters in refined edible oils: A review on formation, occurrence, analysis, and elimination methods. *Comprehensive Reviews in Food Science and Food Safety*, 16(2), 263–281.

[16] da Silva, L. A., Pezzini, B. R., & Soares, L. (2015). Spectrophotometric determination of the total flavonoid content in *Ocimum basilicum* L. (Lamiaceae) leaves. *Pharmacognosy Magazine*, 11(41), 96.

[17] Dalei, M. K., Dehuri, M., Palai, S., Mohanty, B. N., Kuppusamy, S., & Panda, M. R. (2022). Phytochemical investigation and evaluation of acaricidal activities of *Datura stramonium* fruits against *Rhipicephalus microplus*. *Journal of Environmental Biology*, 43(5), 727–732.

[18] Das, S. K., Samantaray, D., Sahoo, S. K., Patra, J. K., Samanta, L., & Thatoi, H. (2019). Bioactivity guided isolation and structural characterization of the antidiabetic and antioxidant compound from bark extract of Avicennia officinalis L. *South African Journal of Botany*, *125*, 109-115.

[19] Das, B., Saha, P., & Maity, N. (2024). Quantitative estimation of quercetin of some selected edible plants of West Bengal by high-performance thin-layer chromatography densitometry method. *Asian Journal of Pharmaceutical Research and Health Care (AJPRHC)*, 15, 254–261.

[20] Daud, M. K., Nafees, M., Ali, S., Rizwan, M., Bajwa, R. A., Shakoor, M. B., Arshad, M. U., Chatha, S. A., Deeba, F., Murad, W., & Malook, I. (2017). Drinking water quality status and contamination in Pakistan. *BioMed Research International*, 2017(1), 7908183.

[21] Devi, D., Devakadacham, S. R., Saveetha, D., & Manikandan, J. (2024). Enhancing insect species identification in agriculture using fusion BiLSTM network and VGG-16 CNN architecture. In *2024 International Conference on Advances in Computing, Communication and Applied Informatics (ACCAI)* 1–6.

[22] Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, 22(3), 296–302.

[23] Eden, T., Burns, E., Freccero, P., Renner, L., Paintsil, V., Dolendo, M., Scanlan, T., Khaing, A. A., Pina, M., Islam, A., & Chunda-Liyoka, C. (2019). Are essential medicines available, reliable and affordable in low-middle income countries? *Journal of Cancer Policy*, 19, 100180.

[24] Gancar, M., Kurin, E., Bednarikova, Z., Marek, J., Mucaji, P., Nagy, M., & Gazova, Z. (2020). Amyloid aggregation of insulin: An interaction study of green tea constituents. *Scientific Reports*, 10(1), 9115.

[25] Górecki, T., Martos, P., & Pawliszyn, J. (1998). Strategies for the analysis of polar solvents in liquid matrixes. *Analytical Chemistry*, 70(1), 19–27.

[26] Gope, E. R., Begum, S. M., Anisetti, P. P., Kasa, G. G., Eedarada, V. G., Nalli, J., & Thummidi, R. S. (2024). A review of principles, applications, and recent developments in HPTLC and HPLC. *Journal of Pharma Insights and Research*, *2*(6), 056-064.

[27] Han X, Wang R, Zhou Y, Fei L, Sun H, Lai S, Saadatpour A, Zhou Z, Chen H, Ye F, Huang D. (2018). Mapping the mouse cell atlas by microwell-seq. Cell., 172(5):1091-107.

[28] Holmberg, K. (2007). Organic reactions in microemulsions. *European journal of organic chemistry*, *2007*(5), 731-742.

[29] Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M. C. B., & Rahu, N. (2016). Oxidative stress and inflammation: What polyphenols can do for us? *Oxidative Medicine and Cellular Longevity*, 2016, 9.

[30] Hwang, E. S., & Nhuan, D. T. (2014). Antioxidant contents and antioxidant activities of hot-water extracts of aronia (*Aronia melanocarpa*) with different drying methods. *Korean Journal of Food Science and Technology*, 46(3).

[31] Izhar, M. P., Hafeez, A., Kushwaha, P., & Simrah. (2023). Drug delivery through niosomes: A comprehensive review with therapeutic applications. *Journal of Cluster Science*, 21, 1–7.

[32] Jahan, A., Akhtar, J., Badruddeen, Jaiswal, N., Ali, A., & Ahmad, U. (2022). Recapitulate genistein for topical applications including nanotechnology delivery. *Inorganic and Nano-Metal Chemistry*, 52(9), 1306–1317.

[33] Jha, M., Sharma, V., & Ganesh, N. (2012). In vitro antioxidant and cytotoxicity assay of Pistia Stratiotes L. against B16f1 and B16f10 melanoma cell lines. In *Chemistry of Phytopotentials: Health, Energy and Environmental Perspectives*.Berlin, Heidelberg: Springer Berlin Heidelberg. 19-23.

[34] Jiang, J., & Jia, X. (2015). Profiling of fatty acids composition in suet oil based on GC–EI-qMS and chemometrics analysis. *International Journal of Molecular Sciences*, 16, 2864–2878.

[35] Julien, M., Hoeffel, J. M., & Flick, M. R. (1986). Oleic acid lung injury in sheep. *Journal of Applied Physiology*, 60(2), 433–440.

[36] Kaboré, B., Koala, M., Ouedraogo, C. R., Belemnaba, L., Nitiema, M., Compaoré, S., Ouedraogo, S., Ouedraogo, N., Dabiré, C. M., Kini, F. B., & Palé, E. (2023). High-performance thin-layer chromatography phytochemical profiling, antioxidant activities, and acute toxicity of leaves extracts of *Lannea velutina* A. Rich. *Rich Journal of Medicinal Chemistry Sciences*, 6, 410–423.

[37] Kalaskar, M., Yele, S. U., Ayyanar, M., Gurav, N., Beldar, V., & Surana, S. J. (2025). Methods of extraction. In *Pharmacognosy and Phytochemistry: Principles, Techniques, and Clinical Applications*, 121–142.

[38] Khan, M. A., Marwat, K. B., Gul, B., Wahid, F., Khan, H., & Hashim, S. (2014). *Pistia stratiotes* L. (Araceae): Phytochemistry, use in medicines, phytoremediation, biogas and management options. *Pakistan Journal of Botany*, 46(3), 851–860.

[39] Kristiningrum, N., Wulandari, L., & Zuhriyah, A. (2018). Phytochemical screening, total phenolic content, and antioxidant activity of water, ethyl acetate, and n-hexane fractions from mistletoe *Moringa oleifera* Lam. (*Dendrophthoe pentandra* (L.) Miq.). *Dendrophthoe pentandra*, 104–106.

[40] Kumar, R. V., Gosipatala, S. B., Kumar, R., Srivastava, D., Singh, V., Suman, K., Tripathi, D. K., Verma, A., Mishra, A., Vishwakarma, K. K., Singh, S. A., Pandey, T., Agarwal, S., Elyies, M., Singh, I., Sah, P. K., Sharma, C., Parag, R., Saxena, P., Raj, A., Tripathi, A., Devi, P., & Poluri, K. M. (2023). Characterization, antioxidant, and antimicrobial properties of mulberry lattices. *ACS Omega*, 8(50), 47758–47772.

[41] Kushwaha, P., Saxena, S., & Shukla, B. (2021). A recent overview on dermatological applications of liposomes. *Recent Patents on Nanotechnology*, 15(4), 310–321.

[42] Lakshita, Jalwal, P., & Shailja. (2025). Exploration of antidiabetic potentials of *Origanum vulgare* plant extract. *Journal of Neonatal Surgery*, 14(13s), 767–783.

[43] Lee, I., & Lee, K. (2015). The Internet of Things (IoT): Applications, investments, and challenges for enterprises. *Business Horizons*, 58(4), 431–440.

[44] Lezoul, N. E., Belkadi, M., Habibi, F., & Guillén, F. (2020). Extraction processes with several solvents on total bioactive compounds in different organs of three medicinal plants. *Molecules*, 25(20), 4672.

[45] Lu, W. I., & Lu, D. P. (2014). Impact of Chinese herbal medicine on American society and health care system: Perspective and concern. *Evidence-Based Complementary and Alternative Medicine*, 2014(1), 251891.

[46] Magiera, A., Czerwińska, M. E., Owczarek, A., Marchelak, A., Granica, S., & Olszewska, M. A. (2022). Polyphenols and Maillard reaction products in dried *Prunus spinosa* fruits: Quality aspects and contribution to anti-inflammatory and antioxidant activity in human immune cells ex vivo. *Molecules*, 27(10), 3302.

[47] RANJAN, S., Chaitali, R. O. Y., & SINHA, S. K. (2023). Gas chromatography–mass spectrometry (GC-MS): a comprehensive review of synergistic combinations and their applications in the past two decades. *Journal of Analytical Sciences and Applied Biotechnology*, *5*(2), 72-85.

[48] Majumder, S., Ghosh, A., & Bhattacharya, M. (2020). Natural anti-inflammatory terpenoids in *Camellia japonica* leaf and probable biosynthesis pathways of the metabolome. *Bulletin of the National Research Centre*, 44, 141.

[49] Malayaman, V., Mohamed, S. S., Senthilkumar, R. P., & Basha, M. G. (2019). Analysis of phytochemical constituents in leaves of *Bhumyamalaki (Phyllanthus debilis* Klein ex Willd.) from Servaroy Hills, Tamil Nadu, India. *Journal of Pharmacognosy and Phytochemistry*, 8(1), 2678–2683.

[50] Mamoona, Nosheen, S., Riaz, S., Shah, S. I., & Shahid, S. (2025). Optimizing extraction methods: The role of solvent polarity in enhancing phenolic content and antioxidant activity in biowaste. *Biomass Conversion and Biorefinery*, 15(11), 16721–16736.

[51] Matic, P., Sabljic, M., & Jakobek, L. (2017). Validation of spectrophotometric methods for the determination of total polyphenol and total flavonoid content. *Journal of AOAC International*, 100(6), 1795–1803.

[52] Mazumder, K., Nabila, A., Aktar, A., & Farahnaky, A. (2020). Bioactive variability and in vitro and in vivo antioxidant activity of unprocessed and processed flour of nine cultivars of Australian lupin species: A comprehensive substantiation. *Antioxidants*, 9(4), 282.

[53] Mizushina, Y., Tanaka, N., Yagi, H., Kurosawa, T., Onoue, M., Seto, H., Horie, T. I., Aoyagi, N., Yamaoka, M., Matsukage, A., & Yoshida, S. (1996). Fatty acids selectively inhibit eukaryotic DNA polymerase activities in vitro. *Biochimica et Biophysica Acta (BBA) – Gene Structure and Expression*, 1308(3), 256–262.

[54] Mokaizh, A. A. B., Nour, A. H., Alazaiza, M. Y. D., Mustafa, S. E., Omer, M. S., & Nassani, D. E. (2024). Extraction and characterization of biological phytoconstituents of *Commiphora gileadensis* leaves using Soxhlet method. *Processes*, 12(8), 1567.

[55] Nawaz, H., Shad, M. A., Rehman, N., Andaleeb, H., & Ullah, N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Brazilian Journal of Pharmaceutical Sciences*, 56, 17129.

[56] Netshiluvhi, T. R., & Eloff, J. N. (2016). Effect of water stress on antimicrobial activity of selected medicinal plant species. *South African Journal of Botany*, 102, 202–207.

[57] Nguyen, L. T. H. (2022). Signaling pathways and targets of natural products in psoriasis treatment. *Exploration of Medicine*, 3, 345–367.

[58] Niki, E., Kawakami, A., Saito, M., Yamamoto, Y., Tsuchiya, J., & Kamiya, Y. (1985). Effect of phytyl side chain of vitamin E on its antioxidant activity. *Journal of Biological Chemistry*, 260(4), 2191–2196.

[59] Orak, H. H., Bahrisefit, I. S., & Sabudak, T. (2019). Antioxidant activity of extracts of soursop (*Annona muricata* L.) leaves, fruit pulps, peels, and seeds. *Polish Journal of Food and Nutrition Sciences*, 69(4), 359–366.

[60] Peana, A. T., D'Aquila, P. S., Panin, F., Serra, G., Pippia, P., & Moretti, M. D. (2002). Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. *Phytomedicine*, 9(8), 721–726.

[61] Kumar, A., Gupta, M., Gupta, L. M., Kour, S., & Choudhary, P. (2025). Evaluation of growth, yield attributes, and yield of wheat varieties under Terminalia chebula trees. *Frontiers in Sustainable Food Systems*, *8*, 1417400.

[62] Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S., et al. (2014). Oxidative stress, prooxidants, and antioxidants: The interplay. *BioMed Research International*, 2014, 761264.

[63] Rokaya, M. B., Bashyal, S., & Timsina, B. (2023). Phytochemistry and ethnopharmacological importance of *Pistia stratiotes* L. (Hemsl) A. Grey (Araceae). In *Aquatic Medicinal Plants*, 133–162.

[64] Sahu, R., & Saxena, J. (2013). Screening of total phenolic and flavonoid content in conventional and non-conventional species of *Curcuma*. *Journal of Pharmacognosy and Phytochemistry*, 2(1).

[65] Saito, Y., Takao, H., Tani, T., Nonoyama, T., Takatori, K., Homma, T., Nagaya, T., & Nakamura, M. (2004). Lead-free piezoceramics. *Nature*, 432(7013), 84–87.

[66] Sarfraz, M., Alam, F., Din, K. M., Malik, S., & Quddoos, A. (2024). Pharmacognostic characterization of Dicleptera chinensis by scanning electron microscopy, light microscopy, and analytical techniques. *Microscopy Research and Technique*, *87*(2), 279-290.

[67] Shah, S. M., Ayaz, M., Khan, A. U., Ullah, F., Farhan, Shah, A. U., Iqbal, H., & Hussain, S. (2015). 1,1-Diphenyl, 2-picrylhydrazyl free radical scavenging, bactericidal, fungicidal and leishmanicidal properties of *Teucrium stocksianum*. *Toxicology and Industrial Health*, 31(11), 1037–1043.

[68] Shi M, Lam TT, Hon CC, Hui RK, Faaberg KS, Wennblom T, Murtaugh MP, Stadejek T, Leung FC. Molecular epidemiology of PRRSV: a phylogenetic perspective. (2010).Virus research, 154(1-2):7-17.

[69] Shi, L., Zhao, W., Yang, Z., Subbiah, V., & Suleria, H. A. (2022). Extraction and characterization of phenolic compounds and their potential antioxidant activities. *Environmental Science and Pollution Research*, 29(54), 81112–81129.

[70] Siddiqui, Z., Khan, M. I., Akhtar, J., & Ahmad, M. (2022). Multifunctional role of *Phyllanthus acidus* L. as a therapeutic agent for management of diabetes and associated complications: A review. *Biomedical and Pharmacology Journal*, 15(4), 1821–1831.

[71] Sosa, V., Moline, T., Somoza, R., Paciucci, R., Kondoh, H., & LLeonart, M. E. (2013). Oxidative stress and cancer: An overview. *Ageing Research Reviews*, 12(1), 376–390.

[72] Sudirman, S., Herpandi, R., Lestari, S., Harma, M., & Aprilia, C. (2024). Effects of extraction temperature on bioactive compounds and antioxidant activity of yellow velvetleaf (*Limnocharis flava*) and water lettuce (*Pistia stratiotes*) leaf extract. *Food Research*, 8(1), 136–139.

[73] Sugumar, J. K., & Guha, P. (2025). Evaluation of the changes in physicochemical and functional characteristics of leaves of Solanum nigrum under different drying methods. *Measurement: Food*, *17*, 100211.

[74] Sureshkumar, V. (2021). Phytochemical screening and thin layer chromatography profiling of various extracts of *Achyranthes aspera* and *Cissus quadrangularis*. *Journal of Phytopharmacology*, 10(4), 225–229.

[75] Tassakka, A. C., Sumule, O., Massi, M. N., Manggau, M., Iskandar, I. W., Alam, J. F., Permana, A. D., & Liao, L. M. (2021). Potential bioactive compounds as SARS-CoV-2 inhibitors from extracts of the marine red alga *Halymenia durvillei* (Rhodophyta) – A computational study. *Arabian Journal of Chemistry*, 14(11), 103393.

[76] Taylor, R. B., & Sapozhnikova, Y. (2022). Assessing chemical migration from plastic food packaging into food simulant by gas and liquid chromatography with high-resolution mass spectrometry. *Journal of Agricultural and Food Chemistry*, 70(16), 4805–4816.

[77] Tyagi, T., & Agarwal, M. (2017). Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) Solms. *Journal of Pharmacognosy and Phytochemistry*, 6(1), 195–206.

[78] Uchegbu, R. I., Bako, S. S., Olehi, L. C. N., & Achinihu, I. O. (2015). GC/Ms Analysis and identification of phytochemicals present in the fruits of Mormodica balsamina Linn. *IOSR J. Appl. Chem*, *8*, 39-42.

[79] Unachukwu, U. J., Ahmed, S., Kavalier, A., Lyles, J. T., & Kennelly, E. J. (2010). White and green teas (*Camellia sinensis* var. *sinensis*): Variation in phenolic, methylxanthine, and antioxidant profiles. *Journal of Food Science*, 75(6), C541–C548.

[80] Vambe, M., Naidoo, D., Aremu, A. O., Finnie, J. F., & Van Staden, J. (2021). Bioassay-guided purification, GC-MS characterization and quantification of phyto-components in an antibacterial extract of Searsia lancea leaves. *Natural product research*, *35*(22), 4658-4662.

[81] Yenet, A., Nibret, G., & Tegegne, B. A. (2023). Challenges to the availability and affordability of essential medicines in African countries: A scoping review. *ClinicoEconomics and Outcomes Research*, 15, 443–458.

[82] Zeb, A. (2020). Concept, mechanism, and applications of phenolic antioxidants in foods. *Journal of Food Biochemistry*, 44(9), e13394.