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RESEARCH ARTICLE

Phytochemical profiling and GC-MS analysis of methanolic leaf extract of *Persicaria hydropiper* (L.) H. Gross: An important ethnomedicinal plant

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Abstract

Medicinal plants are the best source of alternative medicine and are acquiring recognition in the area of pharmacological research for the production of plant-based medicines with improved efficacy. *Persicaria hydropiper* (L.) H-Gross is known worldwide for its use in traditional medicine systems. It possesses numerous therapeutic properties. The present endeavor was taken up to analyze the phytochemical profile and antioxidant activity by DPPH assay of various extracts of *Persicaria hydropiper* leaves. The preliminary phytochemical screening of acetone, methanol, and aqueous extract of *P. hydropiper* leaves showed the presence of important phytochemicals such as phenols, tannins, flavonoids, glycosides, terpenoids, and alkaloids. The quantitative estimation revealed elevated amounts of total phenols, flavonoids, tannins, and total alkaloid content. The antioxidant activity also revealed higher radical scavenging activity by the extracts. The Gas Chromatography-Mass Spectrophotometry assay of methanol extract of *P. hydropiper* leaves detected and identified altogether 54 phytocompounds including 7 major compounds displaying higher peaks such as Andrographolide, 1H-cyclopropa[a] naphthalene, 1a,2,3,5.6,7a7b-octahydro-1,1,7,7a-tetramethyl-[1aR-(1aα,7α,7aα,7bα)-, Cis-Z alpha bisabolene epoxide, 1,8-cyclopentadecadiyne, Aromadendrene oxide (2), Bicyclo[4.1.0]heptane-3-cyclopropyl 7-hydroxymethyl, (cis) and Alpha-guanine. The concentration of phenols, flavonoids, and tannins revealed in the phytochemical profiling of three extracts might be responsible for the strong antioxidant activity of the extracts and the bioactive compounds identified in the GC-MS investigation exhibit important therapeutic properties required for the treatment of various human diseases. Thus, the medicinal use and properties of *Persicaria hydropiper* may be attributed to the presence of these phytochemical constituents. Further study of the compounds may provide scope for the production of novel drugs with increased efficacy and potency with the ability to cure harmful human diseases.

Keywords

Persicaria hydropiper; bioactive; antioxidant; medicinal plants; phytochemicals

Introduction

The therapeutic properties of medicinal plants may be attributed to the fact that they possess curative properties and expend certain pharmacological

effects that are beneficial for the human body. They have been used for a very long time to reduce human suffering from various diseases. In recent years there has been an increase in the need for drugs obtained from medicinal plants as they are safer to use, easily available, and cost-effective than synthetic medicines (1). These plants have been verified to be the best source of alternative medicines. The backbone of the medicinal properties of plants lies in the broad spectrum of chemical compounds present in their different parts such as leaves, flowers, stems, roots, fruits, and bark (2). The chemical compounds commonly known as phytochemicals are bioactive in nature and produce specific physiological actions in the human body. Thus, allowing them to aid in the treatment of many human ailments. Plant-derived substances exhibiting such properties have formed the basic source for the development of modern-day drugs (3). The most important among these substances are phenolic compounds, tannins, and alkaloids (4). The rising demand for phytomedicines by consumers due to their higher efficacy and safety has urged many scientists to carry out elaborate research on the biological activities of medicinal plants.

The phytochemicals are the basis for various physiological effects such as regulation of molecular signal transduction pathways that cause inflammation in the human body. This leads to the prevention of many chronic diseases such as cancer, diabetes, articular rheumatism, obesity, atherosclerosis, skin diseases, aging, cancer, and antioxidant and anti-inflammatory diseases (5). The presence of phytochemicals such as flavonoids, alkaloids, and tannins exhibited potent cytotoxic effects (6). Phenolic compounds act as natural antioxidants by neutralizing the deleterious effects of free radicals and improving carbohydrate metabolism (7). Tannins possess therapeutic properties such as wound healing, anti-inflammatory, antioxidant, and act as analgesic agents (8). They also exhibit anticancer properties (9). Flavonoids reduce the risk of cardiovascular diseases and cancer (10). Alkaloids were known to possess analgesic, antispasmodic, and antibacterial properties (11).

The generation of unbalanced free radicals causes abnormal physiological conditions in the human body that lead to cell damage through the degradation of lipids, proteins, and nucleic acids (12). The free radicals are responsible for the onset of many harmful ailments such as cancer, Alzheimer's disease, Parkinson's disease, inflammatory disorders, lipid peroxidation, cardiovascular disease, and diabetes. Antioxidants are the compounds that protect the cells from the damage caused by free radicals (13). The secondary metabolites produced by plants are a good source of natural antioxidants (14). Medicinal plants are long been used as the safest, most effective, and most sustainable source of natural antioxidants, especially the phenolic compounds such as flavonoids, phenolic acids, tannins, and anthocyanins (15) which combat the harmful effects of free radicals by terminating the chain reaction that regulates free radical-mediated oxidative processes (12). Despite the development of many synthetic antioxidants to remediate oxidative stress, natural antioxidants acquired from plants are preferred over synthetic ones. Considering the versatile applications of these substances the quantification and identification of these compounds in medicinal plants is the need of the hour so that they might be utilized for the synthesis of new drugs that may prove beneficial for the human race shortly.

One of the most popular medicinal plants known globally is *Persicaria hydropiper* (L.) H. Gross has a long-standing history of use in the traditional medicine system as a remedial cure for various human ailments (16). It belongs to the family Polygonaceae with the synonyms *Persicaria hydropiper* (L.) Delabre, *Persicaria hydropiper* (L.) Spach, *P. hydropiper* (L.) Opiz, *Polygonum hydropiper* L. and *P. hydropiper* var. projectum Stanford (17, 18). The species is commonly known as marsh-pepper smartweed, marsh-pepper knotweed, or water pepper.

Persicaria hydropiper is an annual plant of about 40-70 cm in height with a decumbent to ascending or erect branched glabrous. The leaves are petiolate having a lanceolate shape and cuneate base, with apex being acute or acuminate and ciliated margin. Inflorescence is either terminal or axillary erect with glabrous peduncles as shown in **Fig 1**. The flowers are white or pink distal obovate perianth and 3-5 in number (19). The plant is indigenous to temperate and tropical Asia including China, India, and Malaysia. It is also reported to be found in Europe, Northern Africa, and Australia. It mostly grows in the wild along water sides and marshes. In India, it is found in Assam, Arunachal Pradesh, and Tripura.

The plant is used traditionally by the Missing women in Assam where they use the dried root powder for terminating pregnancy (20). The Tai-Khamyangs of Assam use the decoction of the leaves to reduce menstrual bleeding and are consumed by pregnant women to retain body energy. The juice of the leaves is given for treating uterine disorders (21). In Arunachal Pradesh, the infusion of the leaves is used to cure colic pain (22). In Tripura, the leaves of *P. hydropiper* are crushed and mixed with black pepper to treat headaches (23). According to earlier reports, the plant is used against many other disorders such as diarrhea, dyspepsia, excessive flow during menstruation, and hemorrhoids either singly or mixed with other herbals (24).

The presence of several bioactive compounds such as flavonoids, sesquiterpenes, and phenylpropanoids exhibiting anticancer, antioxidant, antileukemia, antibacterial, antinociceptive, tyrosinase-inhibiting and aldose reductase inhibitory have been identified and reported earlier in *P. hydropiper* species (25). The pharmacological properties of *Persicaria hydropiper* have been mentioned in several reports published earlier that support its usefulness in traditional medicines. Some of these include antioxidant properties (26), antibacterial (27), antifungal, anthelmintic, antifeedant (28), cytotoxic effect (29), anti-inflammatory (30), oestrogenic and antifertility (31) and neuroprotective activity (32).

Considering the vast potentialities of *Persicaria hydropiper* as an important therapeutic agent, the present endeavor was taken up to investigate further, the phytochemical profile and the antioxidant activity of leaves of the plant in different solvent extracts. GC-MS assay was carried out for the methanol extract of the plant to identify important phytochemical substances existing in the extract. These compounds are highly in demand and might aid in the manufacture of new drugs beneficial for treating human diseases.

Fig.1 Leaves and flowers of *Persicaria hydropiper* (L.) H. Gross plant

Materials and Methods

Collection and identification of plant material:

Leaves of *Persicaria hydropiper* (L.) H. Gross were plucked and collected from Rajapukhuri Shyam village (26° 08´30´N and 93°54´30´E) located in Golaghat district of Assam, India during the month of January 2022. The authentication of the plant was done at the Botanical Society of India, Eastern Circle in Shillong, Meghalaya, India. The accession number for *Persicaria hydropiper* (L.) H. Gross given was 98161. A voucher specimen was preserved at the BSI herbarium, in Shillong, India.

Preparation of plant material in different solvent extracts:

After collection, the leaves of *P. hydropiper* were washed carefully in running water and then dried in shade for 7-8 days. After the drying was done the leaves were crushed by using a mechanical grinder to powdery form that was used for extract preparation. The process of extraction was conducted using Soxhlet's apparatus in solvents of different polarity viz., acetone, methanol, and water. 5gm of the dried material was first placed into the thimble and extracted using 500ml of different solvents separately. The process continues till the solvent color disappears. Then each extract was heated in a water bath at 30-40°C until the solvent evaporated. The dry extract was kept in the refrigerator for further analysis.

Preliminary phytochemical screening:

The preliminary screening for the presence of important phytochemicals such as phenols, flavonoids, carbohydrates, glycosides, proteins, saponins, and terpenoids was carried out following the standard procedures described by Harborne (33) and Trease and Evans (34) and Sofowara (35)

Quantification of Total Phenolic Content (TPC):

The Folin- ciocalteu assay (36) was used for determining the total phenolic content. Gallic acid was used as the standard. The stock solution was made by adding and mixing 10mg of gallic acid in 100ml distilled water. Five reference standards with different concentrations of 20, 40, 60, 80, and 100 µg/ml were prepared each in separate test tubes. 2 ml of distilled water was then added to each test tube. To each mixture, 0.3ml of Folin- ciocalteu reagent was added and the mixture was allowed to stand for 5 minutes. After this, an amount of 0.8ml 20% sodium carbonate solution was added and the volume of each mixture was finally made to 5ml by adding distilled water. Similarly, 1ml of acetone, methanol, and water extracts of *P. hydropiper* leaves were prepared by mixing them with the same reagents in separate test tubes. The final volume of each extract was made to 5ml with distilled water. The absorbance of each extract and stock was measured after 30 minutes at 765nm using a UV-VIS spectrophotometer in triplicates. The final calculations were done in terms of mg Gallic acid equivalent or mg (GAE)/gm of plant extract.

Quantification of Total Flavonoid Content (TFC):

TFC was measured by using the aluminum chloride method (37) in which the standard used was quercetin. The stock solution of the standard was formed by dissolving 0.1gm quercetin in 100ml distilled water. Five standard references of different concentrations of 20, 40, 60, 80, and 100µg/ml in separate test tubes were made. To 0.5ml of each solution, 1.5ml methanol was added. Then 0.5 ml of 10% aluminum chloride was added to each solution. 0.1ml of sodium acetate was mixed and the volume of each solution was made to 5ml finally using distilled water. For preparation 1mg of each extract was dissolved in 1ml methanol in separate test tubes. 0.5ml of each sample was then mixed with the same reagents as mentioned above. Each mixture (standard/sample) was shaken thoroughly and allowed to rest for 30 minutes at normal temperature. The absorbance of standard and samples was measured at 415nm. All the readings were taken in triplicates. The results were calculated as mg Quercetin equivalent (QE)/gm of plant extract.

Quantification of Total Tannin Content (TTC):

Total tannins were evaluated by following the method illustrated by Sudha *et al.,* 2018 (38). The standard used was tannic acid. 1mg of tannic acid was dissolved in 1 ml of distilled water for the stock solution. From this again 1ml stock was mixed with 9ml of distilled water to form a diluted solution. Five different reference standards of concentrations 20, 40, 60, 80, and 100 µl were prepared in test tubes each separately. To each reference, 2ml of 0.1M ferric chloride solution mixed in 0.1N hydrochloric acid and 0.008M potassium ferrocyanide was added. For the preparation of the sample in a conical flask 50mg of each

extract was mixed with 50ml distilled water. Using a mechanical shaker each mixture was shaken for 1 hour and then filtered into a volumetric flask of 50ml. By adding distilled water, the mixture was added to the final volume of the conical flask. 1 ml of each filtrate was mixed with 4 ml of distilled water. Then the same reagents were added to each mixture as mentioned above. The combination was shaken carefully and made to rest for 30 minutes at normal temperature. The absorbance of each standard and sample was taken at 700nm using a UV-VIS spectrophotometer. The final results were calculated as mg tannic acid equivalent (TAE)/gm of dried plant extract.

Quantification of Total Alkaloid Content:

Total alkaloids were determined by using Dragendorff's reagent method (39). Here berberine chloride was the standard. The stock solution was prepared at a concentration of 1mg/ml of distilled water. From the stock five reference standards of concentration 20, 40, 60, 80, and 100 µg/ml were prepared. Sample preparation was done at a concentration of 1g/ml in distilled water. Then 0.5ml of each standard and sample was taken in separate test tubes and the pH of each was maintained with dilute hydrochloric acid to 2-2.5. To each of the above mixtures, 2ml of Dragendorff's reagent was added. The precipitate that was formed was centrifuged. After complete centrifugation, the centrifugate was decanted carefully. The precipitate was again washed with alcohol and filtered. After filtration the residue was collected and 2ml disodium sulphide solution was added. The precipitate was again centrifuged. The residue obtained was mixed with 2ml concentrated nitric acid and warmed. Resulted mixture was diluted in a 10ml flask to the final volume with distilled water. From this 1ml of each solution was then taken out separate test tubes and mixed with 5ml thiourea solution. The absorbance was read at 435nm.

Antioxidant activity of the plant samples:

The antioxidant activity of the plant samples was determined by the DPPH (Diphenyl-1-picrylhydrazyl) assay described by Mensor *et al.,* 2001(40).

Principle: When DPPH solution is added to the plant extracts, the purple color of DPPH undergoes discoloration due to the reaction between the antioxidant molecules present in the plant extracts and the DPPH. The degree of discoloration of purple to yellow is measured at 517nm, which is a measure of the scavenging potential of plant extract. Ascorbic acid is used as standard.

DPPH solution was prepared by dissolving 4mg DPPH in 100 ml methanol. Standard ascorbic acid was prepared at a concentration of 0.1mg/ml methanol. Samples were prepared at a concentration of 20-100 µg/ml. The test solution was prepared by adding 1 ml of sample or standard to 2 ml of DPPH solution in separate test tubes for different concentrations. The mixture was then shaken vigorously and incubated for 30 minutes in the dark at room temperature. The absorbance of each mixture and standard and the control (2 ml methanol added to 2 ml DPPH solution) was measured at 517nm by a UV- VIS spectrophotometer. The percentage of scavenging activity of the samples is calculated using the following formula:

Eqn.1 **% Inhibition activity=** {(Ac-As)/Ac} × 100

Where Ac= Absorbance of control

As= Absorbance of sample

The absorbance of the samples was compared with that of the control and the standard (Ascorbic acid). The IC_{50} value of samples (concentration of sample required to inhibit 50% of DPPH free radical) was calculated using the Log dose inhibition curve. The lower the absorbance of the reaction mixture indicated higher free radical activity.

Statistical analysis

The results were expressed in terms of mean ± standard deviation. All the data are presented in the form of mean values of triplicate measurements obtained from three separate readings.

GC-MS (Gas chromatography-mass spectrometry) assay of methanolic extract:

The GC-MS assay for the methanolic extract of *P. hydropiper* leaves was carried out at the Institute of Advanced Studies in Science and Technology (IASST), Guwahati, Assam. The analysis was conducted in a combined gas chromatography-mass spectrophotometer system (2010 Plus/TQ8030 model) with a length of 30.0m, 0.25mm diameter, and the thickness of the column was 0.25µM DB-5-MS. Helium was used as the carrier gas. The pressure of the system was 65.9 kPa adjusted to column velocity flow at a rate of mL/min.

Other conditions are volume of the sample injected was 1µL and in splitless mode. The injection temperature was 260°C the temperature of the ion source was 200°C. The mass scan range was covered from 50-1000 m/z. The details of the programmed temperature were as follows – starting column temperature was 80°C which was held for 2 min then increased by 5°C and raised to 230°C. It was again held for 5 minutes and further increased by 3°C raised to 280°C and lastly held for 1 min. The running time of the program was 54.67 minutes.

The data obtained was processed through GC-MS Software Post-run analysis. For identification of the compounds detected in the extract were obtained from the National Institute of Standard and Technology (NIST 11) library and the PESTEI -3 library.

Results and Discussion

Preliminary screening of phytoconstituents:

The results of the preliminary screening of phytochemicals of the acetone, methanol, and water extracts of *P. hydropiper* leaves are presented in **Table 1**. Results revealed that important phytocompounds such as phenols and tannins, flavonoids, carbohydrates, glycosides, terpenoids, and alkaloids were detected to be present in all three extracts of the plant. The results of our study showed that *P. hydropiper* leaves are rich in phenols, flavonoids, tannins, and alkaloids. This is in agreement

with the presence of tannins and flavonoids in the leaves of *P. hydropiper* as reported earlier by Nasir *et al.,* 2020 (41). Saponins were negative in the aqueous extract and proteins were absent in the three extracts.

Table 1: Preliminary phytochemical analysis of acetone, methanol, and water extract of *P. hydropiper* leaves

Phytoconstituents	Tests	Acetone	Methanol	Water
Phenols and Tannins	$FeCl_3$ (Ferric chloride) test	+	+	+
Flavonoids	Shinoda test	-	-	-
	Alkaline reagent test	+	+	+
	Sulphuric acid test	-	-	+
Carbohydrates	Molisch's test	+	+	-
	Fehling's test	-	+	-
Proteins	Ninhydrin test	-	-	-
Saponins	Foam test	+	+	-
Glycosides	Keller-Killani test	+	+	+
	Salkowski test	-	-	+
Terpenoids	Chloroform test	+	+	-
Alkaloids	Mayers test	+	+	-
	Wagners test	-	+	+

"+" indicates present "-" indicates absent

Estimation of Total phenols:

The results of quantitative estimation of phytochemicals including total phenolic, flavonoid, tannin, and alkaloid contents revealed in the acetone, methanol, and aqueous extracts of *P. hydropiper* leaves are presented in **Table 2**. Results showed that *P. hydropiper* leaves are rich in phenol content. This finding is in agreement with the studies conducted by Zulfiker *et al.,* (42) and Mihaylova *et al.,* (43). However, the maximum phenolic content was found in the acetone extract with 110.43±1.00 mg GAE/gm dry weight followed by the methanol extract with 77.65±2.34mg/gm GAE. The previous studies reported higher phenol content in the methanolic as reported by Zulfikre *et al.,* (42), and in the tincture extract reported by Mihaylova *et al.,* (43) of the plant. Phenols are a group of potential immuno-stimulating, compounds that exhibit biological activities such as anticancer, antiviral, antiallergic, and antioxidant activities (44). They are present in most medicinal plants and are used widely in phytotherapy.

Estimation of Total flavonoids:

A similar tendency was observed in the quantification of flavonoid content. The acetone extract revealed the maximum yield of flavonoids with 166.00±2.92 mg QE/gm dry weight and the least amount in the aqueous extract with 27.01±2.94 mg QE/gm dry weight. In contrast to the present study, Zulfikre *et al.,* 2011 reported the highest flavonoid content in the ethanol extract with 589.33±12.83 mg QE/g dry weight followed by water extract with 424.17±36.67 mg QE/gm dry weight. The higher flavonoids were detected in the ethanol tincture extract of the plant that ranged between 3766.27±118.25-6909.66±66.03 µM QE/gm dry weight as reported by Mihaylova *et al.,* 2019. Flavonoids exhibit various important biological activities such as anti-inflammatory, anti-ulcer, anti-hepatotoxic, antiviral, antiallergic, and anti-cancer (45). Flavonoids also act as antioxidant agents scavenging oxidizing molecules such as singlet oxygen and various other free radicals thus, aiding in the prevention of cancer and cardiovascular diseases in humans. Flavonoids possess anti-oxidative and mucosal protective effects (46). The higher amounts of flavonoids in different solvent extracts of the *P. hydropiper* plant revealed in our results support previous studies conducted by Yang *et al.,* 2011 (47).

Total Tannins:

Regarding the total tannins content, results revealed maximum content in the acetone extract with 10.90±0.33 mg TAE/gm dried material followed by the methanol extract with 8.32±0.22 mg TAE/gm dry weight. This finding is in agreement with the study conducted by Nasir *et al.,* 2020. Tannins display anticancer and anti-inflammatory properties and are used to treat ulcerated tissues (48, 49). The presence of tannins in *P. hydropiper* leaves shows that the plant may be used as a treatment against cancer, inflammation, and ulcerated tissues.

Total alkaloids:

In the present investigation, the highest concentration of alkaloid was found in the aqueous extract with 55.13±0.64 µg BE/ml plant material while the least amount was revealed in the acetone extract (23.85±0.66 µg BE/ml sample). While, in a similar study conducted by Nasir *et al.,* 2020, alkaloid content was reported to be higher in the solvent extract of the stem than in the leaf extract of *P. hydropiper*. Alkaloids are anti-analgesic, antispasmodic, and antibacterial agents (50) and show potent activity against gastric ulcers (51).

Thus, the elevated amounts of potentially bioactive compounds revealed in the current report possibly validate the worldwide use of *P. hydropiper* in traditional medicine systems due to its numerous medicinal properties such as anti-inflammatory, neuroprotective, antioxidant, anti-microbial, and anti-infertility among others.

Table 2: Quantitative determination of phytoconstituents in acetone, methanol and aqueous extract of *P. hydropiper* leaves

Parameters	Acetone	Methanol	Aqueous
Total phenolic content (mg GAE/gm)	110.43±1.00	77.65±2.34	21.76±1.52
Total flavonoids content (mg QE/gm)	166.00±2.92	117.01±1.52	27.01±2.94
Total tannin content (mg TAE/gm)	10.90±0.33	8.32±0.22	5.74±0.24
Total alkaloid content (mg BE/ml)	23.85±0.66	41.43±1.82	55.13±0.64

Antioxidant activity by DPPH assay:

In the DPPH free radical scavenging assay ascorbic acid is used as the standard. It exhibited 91.84% of inhibition at 100µg/ml with an IC_{50} value of 6.10µg/ml. The IC_{50} value is the concentration at which free radical (DPPH) is inhibited to 50%. Thus, the lower the IC_{50} value, the higher will be the free radical inhibition activity. Ascorbic acid is used as a standard and the highest inhibition % is found at 100 µg/ml concentration (91.84%). The IC_{50} value was found to be 6.10.

The DPPH free radical scavenging activities of three solvent extracts of *P. hydropiper* and the standard ascorbic acid are presented in Table 3. The IC_{50} values are also depicted in the table. In the present study, the acetone extract showed the highest free radical scavenging activity of 82.26±1.39 at 100µg/ml with an IC_{50} value of 19.81µg/ml followed by the methanolic extract and aqueous extract. Similar results were reported by Nasir *et al.,* 2020 where the acetonic extract of the stem (IC_{50} 1.59mg/ml) and leaves (IC_{50} 2.94mg/ml) showed maximum activity than the ethanolic extract. In contrast, Sharif *et al.,* 2013 (52) reported that the ethanol extract of the plant showed the highest antioxidant activity with an IC_{50} value of 12.21µg/ml.

Antioxidants are compounds that protect the damage of cells from free radicals by preventing oxidative stress. Plants produce numerous antioxidant compounds in the form of secondary metabolites that protect against free radicals and diseases caused by them. Medicinal plants exhibiting antioxidant properties are mainly due to the presence of phenols and flavonoids as the principal components that can scavenge free radicals (53). Phenolic compounds such as phenolic acids, flavonoids, tannins, and stilbenes are mostly responsible for the antioxidant activity of medicinal plants, thereby, preventing the onset of many chronic diseases such as cancer, diabetes, cardiovascular problems, and aging (54, 55). Thus, in the present investigation, it was found that the acetone extract of *P. hydropiper* leaves displayed the highest antioxidant potential which may be attributed to the presence of elevated amounts of phenols, flavonoids, and tannins in the acetone extract revealed in the study.

Gas chromatography-mass spectrometry (GC-MS) analysis:

Gas chromatography- Mass spectroscopy (GC-MS) is a combined technique used in the analysis and identification of biologically active compounds present in plant extracts (56). It plays a key role in the analysis of unknown compounds of plant origin.

Table 3: Antioxidant activities of different solvent extracts of *P. hydropiper*

Concentration (µg/ml)	% Inhibition of solvent extracts			
	Ascorbic acid	Acetone	Methanol	Aqueous
20	56.36±1.39	48.92±1.40	33.04±1.62	30.61±1.29
40	65.30±0.86	57.86±1.82	37.26±1.29	39.69±0.77
60	73.46±1.31	70.17±0.85	46.07±1.42	46.06±1.42
80	84.47±1.05	76.97±0.86	60.51±1.34	58.22±1.18
100	91.84±0.98	82.26±1.39	66.88±1.94	66.52±1.50
IC_{50}(µg/ml)	6.10	19.81	62.46	63.93

The results of GC-MS analysis obtained in the present study revealed altogether 54 compounds detected and identified in the methanolic extract of *P. hydropiper* leaves including both major and minor compounds presented in Table 4. The compounds displaying higher peaks are the major compounds presented in Table 5 along with their retention time, peak number, peak area, molecular weight formula, and chemical structure. The chromatogram of the GC-MS assay is displayed in Fig. 2. Each of the major bioactive compounds identified exhibited several potential therapeutic properties.

The first major phytocompound revealed in the assay is Andrographolide which displayed the highest peak of 10.73% at a retention time of 25.038 mins. Andrographolide is a labdane diterpenoid and is a main bioactive constituent found in various parts of *Andrographis paniculata* (57). It has been detected for the first time in the methanolic leaf extract of *Persicaria hydropiper*. It exhibits a broad range of therapeutic applications such as immunostimulatory (58), antibacterial, anti-tumor, anti-diabetic, anti-malarial, hepatoprotective (59), antioxidant (60), anti-HIV effects (61), anti-inflammatory, anti-platelet aggregation and anti-cancer (62). Quite a few reports are available supporting the use of andrographolide as an anti-cancer agent (63, 64, 65).

Fig 2: GC-MS chromatogram of methanolic extract of *P. hydropiper* leaves

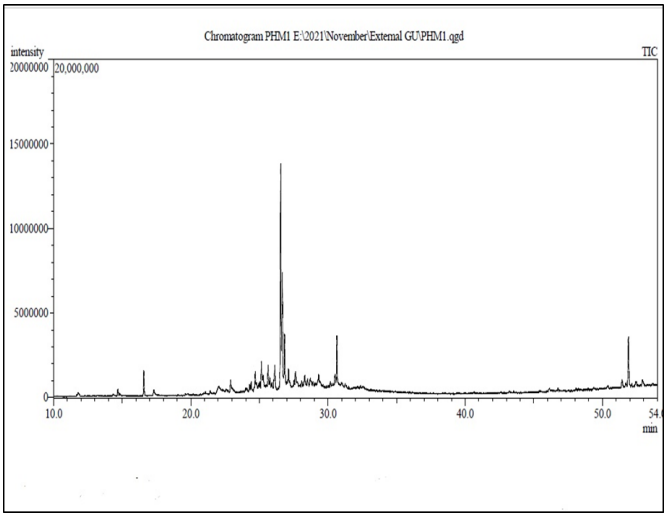


Table 4: Phytochemicals detected in the GC-MS analysis of methanol extract of *P. hydropiper* leaves

PEAKS	RETENTION TIME	NAME	AREA%	HEIGHT %
1	11.78	Isobornyl thiocyanoacetate	0.17	0.1
2	14.687	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1.alpha.,4.alpha.,4a.alpha.,10a.alpha.)-	0.36	0.35
3	16.574	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]	1.19	1.43
4	17.275	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	0.11	0.2
5	17.31	1,4-dihydroxy-p-menth-2-ene	0.16	0.24
6	21.047	(+-)-5-(1-Acetoxy-1-methyl ethyl)-2-methyl-2-cyclohexene-1-one semicarbazone	0.07	0.09
7	21.413	Naphthalene, decahydro-1,6-bis(methylene)-4-(1-methyl ethyl)-, (4.alpha.,4a.alpha.,8a.alpha.)-	0.07	0.1
8	22.063	2,2,4-Trimethyl-3-pentanol	0.24	0.17
9	22.185	Desulphosinigrin	0.08	0.08
10	22.495	Calarene epoxide	0.03	0.05
11	22.619	3-Oxatricyclo[20.8.0.0(7,16)]triaconta-1(22),7(16),9,13,23,29-hexaene	0.18	0.13
12	22.715	Ledene oxide-(I)	0.06	0.11
13	22.745	Methyl 8,10-octadecadiynoate	0.02	0.06
14	22.908	Bicylo[4.1.0]heptane, 7-bicyclo[4.1.0]hept-7-ylidene-	0.54	0.65
15	22.98	Androstenediol	0.15	0.23
16	23.095	10-Undecenoic acid, propyl ester	0.12	0.07
17	24.003	2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	0.2	0.23
18	24.102	Verrucarol	0.25	0.21
19	24.185	Bicyclo[4.3.0]nonane, 2,2,6,7-tetramethyl-7-hydroxy-	0.03	0.07
20	24.285	Doconexent	0.35	0.41
21	24.34	Benzene, 1-cyclopropyl-2-nitro-	0.05	0.14
22	24.404	Caryophyllene oxide	0.38	0.5
23	24.605	Spiro[cyclopropane-1,8'(1H')[3a.6]methano[3ah]cyclopentacycloocten]-10'-one, octahydro-, (3'as,6'R,9'ar)-	0.21	0.24
24	24.704	cis-Z-.alpha.-Bisabolene epoxide	0.98	1.14
25	24.776	1-Naphthalenecarboxylic acid, decahydro-1,4a-dimethyl-6-methylene-5-(3-methyl-2,4-pentadienyl)-, methyl ester, [1S-[1.alpha.,4a	0.56	0.5
26	24.948	Andrographolide	7.18	6.7
27	25.038	Andrographolide	10.73	12.95
28	25.142	Andrographolide	3.14	3.14
29	25.276	1,8-Cyclopentadecadiyne	1.27	0.9
30	25.445	3-Isopropyltricyclo[4.3.1.1(2,5)]undec-3-en-10-ol	0.33	0.28
31	25.511	.alpha.-Guaiene	0.32	0.34
32	25.55	Andrographolide	1.41	1.44
33	25.64	trans-1-Chloro-1,3-dimethylsilacyclohexane	0.19	0.28
34	25.782	Aromadendrene oxide-(2)	0.84	0.74
35	25.903	10-12-Pentacosadiynoic acid	0.53	0.45
36	26.057	3-Tetradecyne	0.58	0.61
37	26.123	Andrographolide	1.44	1.43
38	26.305	Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-	0.05	0.08
39	26.33	4,2,7-Ethanylylidenecyclopenta[b]pyran-9-one, 2,3,4,4a,7,7a-hexahydro-	0.04	0.06
40	26.37	2-(3,3-Dimethyl-but-1-ynyl)-1,1,3-trimethyl-cyclopropane	0.06	0.11
41	26.45	7-Aminoheptanamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]-	0.1	0.18
42	26.551	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	0.49	0.43
43	26.672	7-Hydroxyfarnesen	0.52	0.56
44	26.831	Bicyclo[4.1.0]heptane,-3-cyclopropyl,-7-hydroxymethyl, (cis)	1.8	1.7
45	26.986	trans-2-Methyl-4-n-butylthiane	0.28	0.38
46	27.126	.alpha.-Guaiene	1.37	1.09
47	27.22	1,3a-Ethano-3aH-indene, 1,2,3,6,7,7a-hexahydro-2,2,4,7a-tetramethyl-, [1R-(1.alpha.,3a.alpha.,7a.alpha.)]-	0.3	0.39
48	27.295	Cyclopentanol, 2-cyclopentylidene-	0.08	0.12
49	27.345	Retinal	0.04	0.06
50	27.405	Methyl hinokiate	0.07	0.05
51	27.531	(-)-Spathulenol	0.29	0.39
52	27.626	Andrographolide	1.07	0.85
53	27.785	Bicyclo[10.1.0]trideca-4,8-diene-13-carboxylic acid (2-hydroxy-4-nitrophenyl)amide	0.34	0.25
54	28.082	5-.beta.-Card-20(22)-enolide, 7-.beta.,8-epoxy-3-.beta.,11-.alpha.,14-trihydroxy-12-oxo-	0.37	0.35

The second major compound identified Bicyclo [4.1.0]heptane-3-cyclopropyl 7-hydroxymethyl, (cis) detected at a retention time of 26.831 mins with a peak area of 1.8%. It is a primary alcohol and has been reported earlier in the essential oils of *P. hydropiper* leaves which exhibited potent enzyme-inhibiting activity (66). It is also reported in the methanolic extract of *Lantana trifolia* showing strong antioxidant potential (67)

Alpha-guanine is another major phytocompound detected at 27.126 min with a peak area percentage of 1.37% is a sesquiterpene. It has been detected previously as a main component in the essential oil of aerial parts of *Anaphalis nubigena* DC. Var. *monocephala* (DC.) C.B. Clarke. It acts as a strong antibacterial agent (68) and possesses anti-inflammatory and enzyme-inhibitory activity (69).

The next major bioactive compound detected is 1,8-cyclopentadecadiyne displaying a peak area of 1.27% at 25.276 mins.

1H-cyclopropa[a] naphthalene, 1a,2,3,5.6,7a7b-octahydro-1,1,7,7a-tetramethyl-[1aR-(1aα,7α,7aα,7bα)- identified at retention time 16.574 min with peak area percentage of 1.19% is a sesquiterpene has been It is reported to show antibacterial activity (70) and detected in the GC-MS analysis of *Causonis trifolia* (L.) (71).

Another important sesquiterpene identified is cis-Z alpha bisabolene epoxide at a retention time of 24.704 mins displaying a peak area percentage of 0.98%. It has been reported earlier as a major compound in the essential oil of *Teucrium polium* (72). It has antioxidant, antibacterial, and anti-inflammatory properties (73).

Aromadendrene oxide 2 with a peak area of 0.84% was detected at a retention time of 25.782 mins. Aromadendrene oxide 2 is an oxygenated sesquiterpene found naturally as an important component in the essential oils of *P. missionis* (Wight) Swingle. It exhibits anticancer properties and induces apoptosis (74).

Novelty of the study

The findings of the GC-MS analysis of methanolic leaf extract of *P. hydropiper* revealed in the current study, were compared with some research papers published recently. Ayaz *et al.,* 2016 (75) reported the compounds 4-methyloxazole, succinimide, pyrocatechol, caryophyllene, vanillic acid farnesol, arachidic acid methyl ester, capsaicin, and myristic acid in the methanol extract of *P. hydropiper*. These compounds exhibited potent antibacterial, antifungal, and insecticidal properties. In contrast, the compounds identified in the present report are quite different from them. Most importantly, the phytocompound andrographolide is identified for the first time in the methanolic extract of *P. hydropiper* leaves. This is a new finding in the present endeavor. Andrographolide possesses a broad spectrum of therapeutical properties that might be useful for the synthesis of novel drugs, especially for the treatment of cancer. Also, compounds such as Alpha-guanine, 1,8-cyclopentadecadiyne, and cis-Z alpha bisabolene epoxide have been detected for the first time in the methanolic extract of *P. hydropiper*.

Another study conducted by Chowdhury *et al.,* 2024 (76) revealed the presence of some major bioactive compounds in the GC-MS analysis of a methanolic extract of the plant such as campesterol, n-hexadecenoic acid, 7. alpha.-methylthiotestosterone acetate, neophytadeine, palmitoleic acid, methyl palmitate, phytol, 2-hydroxy-1-(hydroxymethyl) ethyl ester, 9,12,15-Octadecatrienoic acid and 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol, methyl stearate, aristolene epoxide, stigmasterol, and gamma-sitosterol. These compounds possess antinociceptive, anti-inflammatory, antioxidative, and antidiarrheal properties (77). These compounds belong to the classes of polysaccharides, polyphenols, amino acids, steroids, fatty acid esters, and triterpenoids while the compounds identified in the present study are mostly sesquiterpenes and alcohols that possess vital pharmacological properties.

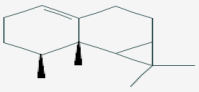
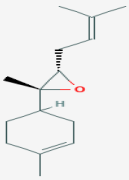
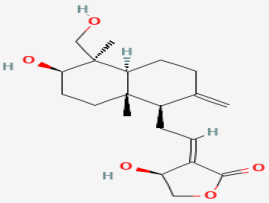
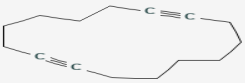
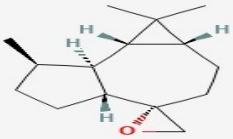
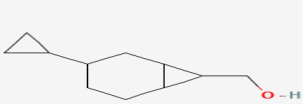
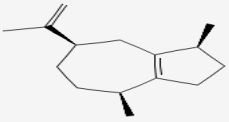
Conclusion

In the present endeavor, our work was mainly focused on the phytochemical profile and antioxidant activity of the acetone, methanol, and aqueous leaf extract of the *Persicaria hydropiper* plant. The higher concentration of phenols, flavonoids, and tannins revealed in the study may be credited for the efficacious antioxidant activity of the plant extracts. The antioxidant properties of the plant can be further utilized in various medicinal therapies for the treatment and prevention of harmful diseases in humans.

Concerning the GC-MS evaluation of the methanolic extract of the leaves that detected a total of 54 phytocompounds including 7 major phytoconstituents among which Andrographolide was reported for the first time in methanolic extract of *P. hydropiper* leaves. It is a bioactive compound that possesses numerous curative properties including anticancer, anti-tumor, anti-diabetic, anti-malarial, hepatoprotective, antioxidant, anti-HIV effects, anti-inflammatory, and anti-platelet aggregation among others. Aromadendrene oxide 2 also displays anti-cancer properties. These two bioactive compounds if isolated from *Persicaria hydropiper* may aid in the remedy of cancer. The other major compounds mostly sesquiterpenes contribute to pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, and enzyme inhibitory.

Thus, the presence of these bioactive phytocompounds may be accountable for the multi-therapeutic application of *P. hydropiper* in the treatment of various human diseases in the traditional medicine system. An insight into the detailed study of the bioactive compounds is essential. This may provide possible scope in the production of new drugs and therapy leading to advancement in the pharmaceutical sector.

Table 5: Major bioactive compounds identified in the GC-MS analysis of methanolic leaf extract of *P. hydropiper*

Sl. No.	Retention Time (min.)	Name of phytocompound	Chemical formula	Molecular wt. (g/mol)	Peak area %	Chemical structure
1.	16.574	1H-cyclopropa[a] naphthalene, 1a,2,3,5.6,7a7b-octahydro-1,1,7,7a-tetramethyl-[1aR-(1aα,7α,7aα,7bα)-	$C_{15}H_{24}$	204.351	1.19	
2.	24.704	Cis-Z alpha bisabolene epoxide	$C_{15}H_{24}O$	220.35	0.98	
3.	25.038	Andrographolide	$C_{20}H_{30}O_5$	350.4	10.73	
4.	25.276	1,8-cyclopentadecadiyne	$C_{15}H_{22}$	202.33	1.27	
5.	25.782	Aromadendrene oxide (2)	$C_{15}H_{24}O$	220.35	0.84	
6.	26.831	Bicyclo[4.1.0]heptane-3-cyclopropyl 7-hydroxymethyl, (cis)	$C_{11}H_{18}O$	166.26	1.8	
7.	27.126	Alpha-guaiene	$C_{15}H_{24}$	204.35	1.37	

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Authors' contributions

RS conceived the research idea. PF collected the specimens, analyzed the data, and carried out the research work under the supervision of RS. RS and PF participated in the design of the study. PF drafted the manuscript and RS revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no competing interest.

Ethical issues: None.

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