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# PHYTOCHEMICAL PROFILING AND MULTIFACETED BIOLOGICAL ACTIVITIES OF *PERSICARIA HYDROPIPER* L.: A PROMISING MEDICINAL PLANT

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**Abstract**

*Persicaria hydropiper* L. is a well-known medicinal plant that has several therapeutic benefits i.e., anti-inflammatory, antioxidant and antimicrobial agent. In the present study, phytochemical profile of *P. hydropiper* was investigated, along with its biological activities like, antibacterial, antifungal, antioxidant, antidiabetic and nematocidal potential via; *In vitro*. Results revealed the presence of various metabolites, like alkaloids, which were present in all partitions. Similarly, other partitions were also rich in flavonoids, terpenoids, glycosides, steroids, carotenoids, tannins, volatile oils and phenolic compounds The mineral contents (mg/100g) were investigated in the following order; K> Na > Ca> Ni >Mg > Fe > Cu > Zn >Pb> Cr>Mn> Cd>Co. Different bacterial and fungal strains growth was investigated, among all, chloroform crude extract showed significant antimicrobial activity ($p$<0.05) against *Escherichia coli*, *Staphylococcus aureus,* and *Streptococcus pneumonia,* i.e., 23 ± 0.31mm, 8 ± 0.75mm, and 9 ± 0.76mm inhibitions, compared to the control. It was noted that ethyl acetate partition was more potent against *E. coli* (24±0.72mm) inhibition and *A. flavus* and *A. fumigatus* 24±2.43mm and 8±2.50mm inhibition, respectively. Additionally, fluorescence microscopy and antioxidant activities revealed prominent findings. Moreover, GC-MS analysis revealed the presence of 61 distinct compounds, with the most prevalent compounds being; Benzene Cyclo Hexyle, Sulfurus Acid, 2 Ethyl Hexyledecyle Ester, 2,4-Di-Tert-Butyl Phenol, Cyclobutanone, 2, 3, 3, 4-Tetra Methyl and Pentaoic Acid and 2-Ethyl Hexyl Ester. Hence, the presence of important phytochemicals, minerals, and resistance to different pathogens demonstrates the importance of *P. hydropiper* in the pharmaceutical industry.

**Key words:** GC-MS; *Persicaria hydropiper;* Biological activities; Antifungal activity; Phytochemicals; Extractions.

## Introduction

Medicinal plants are traditionally used in many countries to prevent and treat many fatal diseases where some pathogenic microbes show very high levels of resistivity (Begum *et al*., 2023). Besides, the latest research shows that some plants, which are used by native people, have bioactive compounds, highly antioxidant, antimicrobial, and important phytochemicals are presented. The different phytochemicals are playing a very important role in the biological activity and treatment of various fetal diseases (Lv *et al*., 2022) Moreover, herbal drugs are commonly used for facing illness and diseases, and for enhancing human overall health (Azmat *et al*., 2023). These studies are usually related to health courses and other medical importance. Uses of traditional medicines are difficult because it composed of different plants (Hussein *et al*., 2020; Saqib *et al*., 2022). Phytochemicals played a key role in the treatment of various fetal diseases e.g., cancer, tumor, diarrhea, dysentery and cardiac vascular diseases, etc. They provide a protective and defensive layer for the human body (Nasir *et al*., 2021; Gao *et al*., 2022). The terpenoids, phenols, and flavonoids are acting as high antioxidant agents. The flavonoids are used as a strong antioxidant in the living body. Phytochemicals are also playing a very important role in the treatment of harmful and other dangerous diseases (Salam *et al*., 2023; Saqib *et al*., 2022).

Reactive oxygen groups (ROS) like lipid peroxyl, superoxide's anion, hydroxyl, singlet oxygen, lipids peroxides, hydrogen peroxide and other radicals are well recognized to react with bimolecular ingredients in living systems, containing protein-lipid and DNA (Khan *et al*., 2021). This could cause different infections like, heart disease, throat infection inflammation, brains disease Parkinson's cancer disease and diabetes myelitis (Nasir *et al*., 2021). The world health organization recorded that 70-80% of people depend on plant medicines for their primary health issue due to being easily accessible and economical (Zahra *et al*., 2017). The *P. hydropiper* is a wild plant that is presents in moist places. The world is wealthy and special natural herbal plants and medicinal plants are currently receiving more concentration the always for the reason that they have the potential countless advantages to the people are to all humans, particularly in the field of medicinal biochemistry (Khan *et al*., 2021). The famous species of *P. hydropiper* are the marsh pepper knotweed, march pepper, and water pepper and it is also called laliao in China, bishkataliorpa karmul in Bangladesh, and daunsenahun in Malaysia. In, their differences are based on the origin region such as the common flora of America and China (Huq *et al*., 2014; He *et al*., 2023).

This study's objective was to evaluate the potential bioactivities of different extracts of *P. hydropiper* for phytochemicals, and mineral analysis by using five different

solvent extraction. We also evaluated its antibacterial, antifungal, antinematode, antioxidants, antidiabetic and GC-MS analysis for the isolation and identification of volatile and semi-volatile compounds. Thus, this herb could be used as a novel source of bioactive phytochemicals.

## Material and Methods

**Preparation of crude extract:** The fresh plants of *P. hydropiper* were collected from the moisture-containing area of district Mansehra, Khyber Pakhtunkhwa (KPK), Pakistan in the months of July 2018. Taxonomist identified the plant and a voucher specimen was submitted to the herbarium of the Department of Botany under no A.K-876. The collected plants were thoroughly cleaned and washed with tap water and dried at room temperature for four weeks in the shade. The dried plants were crushed and ground into a fine powder with the help of a grinder.

**Phytochemical analysis:** Phytochemical analysis to find out the correlation between the biological activities and phytochemicals present in *P. hydropiper* socked in different solvents i.e., Methanol, chloroform, ethyl acetate, n-hexane, and distilled water. To check the phytochemical screening of given or focused sample for the qualitative recognition of terpenoids, saponins, flavonoids, phenol, proteins, alkaloids, glycosides, steroids protein, volatile oils carotenoids and tannins was done by different analytical methods (Rashidi *et al*., 2016).

**Determination of tannins:** The solution of plant extract of 0.5g, 5 mL of distilled water, and 3 mL of 10% lead acetate was prepared. Therefore, the formation of white precipitates was formed which showed the presence of tannins.

**Determination of saponins:** Check out of saponins was determined by the Fourth test. One mL of extract of every fraction of the plant was added to test tubes. The solution was properly mixed and the formations were forth for minutes. Hence, the formation of froth indicated the occurrence of saponins in plants.

**Determination of flavonoids:** 5mL of dilute ammonia mixture was added to 1 mL of every aquas plant extract and then by adding concentrated sulfuric acid yellow color was formed, due presence of yellow color showed that the flavonoids were present.

**Determination of terpenoids:** Two mL of chloroform were mixed with 5 mL of different extracts of *P. hydropiper* plants and then 3 mL of concentrated Sulfuric acid was added for the formation of a layer. The reddish color was produced then it shows the presence of terpenoids.

**Test for alkaloids:** Plant extract 2 mL was mixed with 1.5 mL of 1 % HCL and then added some drops of Wagner's reagent (100 mL of distilled water, 6g potassium, and 2g of iodine). If alkaloids are present, then the formation of reddish-brown precipitates occurred. Alkaloids were determined by Wagner's reagent. If alkaloids are present, then the formation of reddish-brown precipitation occurs.

**Test for glycosides:** The ferric test was performed for the test of Glycosides. 2 mL of Glacial acetic acids was mixed with 1 drop of FeCl3 then added the solution of 1 mL of concentrated sulfuric acid. The formation of brown showed the presence of cardiac glycoside ingredients.

**Identification of steroids:** The steroids were detected by the sulfuric acid test. Two mL of chloroform was dissolved in one mL extract in the test tube when sulfuric acids were added then the formation of reddish precites at interphase was shown the occurrence of Steroids.

**Identification of carotenoids:** Finding carotenoids in plant, test was performed according to Aman *et al*., (2005) 2mL of every extract was taken in a test tube then 8.5% sulfuric acid was added in it. The formation of blue color at the interface of the mixture indicates the occurrence of carotenoids.

**Identification of volatile oils:** To determine the volatile oils in the plants, extract 2 mL of extract was added to the test tube. After that two mL of 85% ethanol and a few drops of ferric acid were added. The formation of dark green precipitation was shown in the occurrence of Volatile Oils.

**Identification of phenols:** To check the phenol ferric analysis was performed. Two mL of plant extract was mixed with a few drops of 10% aqueous ferric chloride Fecl3. The formation of blue color indicates that phenol is present in the plant's extract.

**Biological activities:** Pharmacological and biological activities of the potent plant extract were evaluated i.e., antifungal, antibacterial, antioxidants, anti- nematode, and anti-diabetic *In vitro* as well as *In vivo* anti-diabetic.

**Antibacterial assay:** The various strains of gram-positive and negative bacteria were collected from the micro lab culture of Ayoub Medical College. The well-diffusion method was used for the determination of the antibacterial activity of the samples (Onivogui *et al*., 2015). The media of nutrient agar were used after sterilization in the autoclave. Then inoculate the bacteria on the media. A disc was made by using the cork borer in the center of Petri plates. After loading the samples, the Petri plates were placed in the hybridization oven for 24 hours at 37°C. The inhibition zone of the samples was calculated and measured through the vernier caliper after 24 hours.

The anti-bacterial potential was also determined by disc diffusion methods. The fresh bacterial cultured *Staphylococcus aureus, E. coli, Klebsiella pneumonia, Bacillus subtitles,* and *Streptococcus pneumonia.*

A lawn of refreshed bacterial cultures Staphylococcus aureus, *E. coli*, Klebsiella pneumonia, bacillus subtitles, and streptococcus pneumonia with pre-adjusted seeding concentration was made on nutrient agar plates.

On the seeded plates were placed sterilized filter paper disks saturate and filled out with 5 mL (20 mg/mL DMSO) of each plant extract. The Cefixime (standard) paper disk was used as a positive control while the DMSO disk was used as a negative control. After 24 hours in the incubator

at 37°C the average diameter of the zone of inhibition around the specimen is also present.

Those extracts that have more than 10 mm diameter zone of inhibition, were considered the active plants extract, and further screened for minimum inhibition concentration (MICS) using normal threefold micro broth dilution methodology. The stock of each active plant extract has a different diluted concentration from 100µg/mL to 370 µg /mg in 96 microtiter plates of Mueller Hinton broth media. A reference bacterial strain was prepared i.e., their inoculum to give their specific well inoculum size of approximately 5×102 cfu/mL in each well.

Microtiter plates were then placed at 37°C to be incubated overnight. After incubation, the MIC was determined as the lowest extract concentration inhibiting bacterial strain growth by measuring OD at 600 nm and the triplicate analysis was performed.

**Antifungal assays:** The anti-fungal activity has been determined by disc-diffusion method described by the disc diffusion method was used for the determination of antifungal activity of plant sample extracts. The various known spore strains of fungi such as *Aspergillus flavus* (fcbp-0064), Aspergillus fumigates, Mucor species, and *Fusarium solani* were ground in the 0.03%v/v between tween 20 solution in H2O at Mc Farl and 0.5 turbid standard condition. Then sabour dextrose plates the fungal strain were swabbed.

In this research study the positive control (Clotrimazole 4mg of DMSO) and negative control of DMSO were placed on plates and incubated at 28°C for 24-48 hrs. The test sample extract of 5 mL was impregnated in 20 m/mL of DMSO on sterile filter paper. Around the specimens and monitored disc the Growth of inhibition was measured in average diameter (mm) (Zain *et al*., 2012).

**Antinematicidal activity:** The antinematode activity was performed in the presence of two special media i.e., LB and NGM. The Lb. media are used for the growth of Op 50 bacteria and NGM (nematode growth media) media is especially used for the non-parasitic soil nematodes *C. elegans*.

**Nematocidal Assays:** NGM Plates with enriched *E. coli* (OP-50) media were used for C-elegans culture as a s source of food. The 96-well microtiter plates were used for the nematocidal assays (Polystyrene, flat bottom wells; corning, New York, NY, USA). Different *Persicaria hydropiper* plants extract were used for various concentrations of *C. elegans*. The procedures of the characterization of nematocidal activity were used to maintain the *C. elegans* (Sajid & Azim, 2012).

Every sample of plant extract was dissolved in S medium in different concentrations and put in the 24/48/96 well plates. In the different well plates, the plant extract sample was dissolved in the S-medium in the various concentration. Different developmental stages i.e., Hermaphrodite adult male and larval stages of twenty to 50 *C. elegans* were kept in 100 ul of s medium for twenty 24 hours in a well plate consisting of various concentrations of extraction of plants and 10 ul ×5 µl concentrated overnight culture of *E. coli* maintained in Laura broth and resuspended in s medium. At 20°C the petri plates were incubated for 24 and 48 hours. After 24 and 48 hours of incubation, the survival of *C. elegans* was measured by an inverted microscope (Imico, AC100M-850) and viability was counted i.e., the worm was in motile form.

**Fluorescence microscopy:** Acridine orange (10mg/mL) was used as staining for *C. elegans*. The 2µl S-medium Acridine orange solution of about 500µl was added to NGM plate containing *C. elegans* and incubated at room temperature for 1 hour. Then washed the worms with S-medium and transferred them to NGM plates without ridine orange. Again, incubated at 37°C for 1 hour. Mounted the *C. elegans* on the slide and examined it under the microscope. The nematocidal activity of plant extract was examined at 4X magnification *C. elegans* was observed under the microscope. For this process slides were taken and placed to mount the *C. elegans*. On each edge of the slide, a strip of tap was placed after that add the 2 drops of agarose of 5% on the slide and covered it with the fourth slide in the upward direction. When the agarose was solidified, the upper slide was removed and the agarose pad M9 of 5µl buffer containing worms was placed on it. Then *C. elegans* were examined by using a microscope.

**Anti-diabetic activity**

***In vitro* antidiabetics activity (α-Amylase inhibition assay):** For this assay, the Starch solution was made in 20mM sodium phosphate buffer boiled for 15min in the water bath. Then 1mL of starch solution was mixed with 1mL of extract/acarbose + 1mL of 1mg/mL of α-amylase solution and incubated at 25°C for 3 min. After incubation, the 1mL of DNS reagent was added & heated in the water bath for 15 minutes. After fifteen minutes the absorbance was measured at 540nm.

The Inhibition (%) was calculated by the following formula:

% Inhibition = (1 - As/Ac) ×100 (Kb & Motisariya, 2012)

***In vivo* anti-diabetics activity:** The study was employed on male Albino mice weighted 20–25g (6-8 weeks old) and was purchased from the NIH Islamabad. The suitable/optimized condition was maintained for mice survival. The 12 hours light and dark cycles as well as 22-25°C room temperatures were maintained. All procedures were carried out according to guidelines and used laboratory manual. Ethical approval was obtained from the ethical committee at Hazara University Mansehra, Pakistan.

The anti-diabetic activity of plant extracts was investigated in induced alloxan-induced diabetic mice model. The mice were divided into two main categories. The first category was employed for glycemic study through oral administration of the plant extracts. It is composed of the following 8th groups consisting of five mice in each group (Akhtar *et al*., 2017).

Group 1st consists of normal mice (reference) administrated with 0.5mL normal saline.

Group 2nd consists of diabetic mice dosed with alloxan 150mg/kg with 0.5 mL saline.

Group 3rd composed of diabetic mice (positive control) administrated with glibenclamide (10mg/kg body weight) in 0.5 mL normal saline.

Group 4th composed of diabetic experimental mice dosed with methanolic plant extract dosed with 100mg/kg body weights in 0.5mL normal saline. Five mice were present in each subgroup.

Group 5th: Composed of diabetic experimental mice dosed with chloroform plant extract dosed with 100mg/kg body weights in 0.5mL normal saline. Five mice were present in each subgroup.

Group 6th: Composed of diabetic experimental mice dosed with N-hexanoic extract dosed with 100mg/kg body weights in 0.5mL normal saline. Five mice were present in each subgroup.

Group 7th: Composed of diabetic experimental mice dosed with ethyl acetate extract dosed with 100mg/kg body weights in 0.5mL normal saline. Five mice were present in each subgroup.

Group 8th: Composed of diabetic experimental mice dosed with aquas extract dosed with 100mg/kg body weights in 0.5mL normal saline. Five mice were present in each subgroup.

**DPPH radical scavenging assay:** The 2.2-diphenyl-1-picryl-hydrazyl (DPPH) was used for the determination of antioxidant activity of selected plant extract.

The free radicals DPPH were used as the model of oxidizing agents to be inhibited by the antioxidant plant in the extract. After the preparation of the stock solution, the samples were taken in the test tube for each sample from the stock solution. The mixture was reserved in the dark at normal room temperature.

After the completion of the incubation periods, each sample was run with standard i.e., ascorbic acids and the blank 80% methanol in the spectrophotometer.

The incubation of the samples was used for the absorbance of all the samples, and positive control i.e., ascorbic acid was calculated at 517nm by using a UV spectrophotometer.

The ascorbic acid was used as positive control whereas the DPPH was used as a negative control and 80 % methanol was used as a blank. All the test samples were performed in triplicate. DPPH radical scavenging activity was calculated with the following formula:

$$\text{Inhibition percentage of DPPH activity} = \{AC\text{-}AS/AC\} \times 100$$

While AS, and AC represent.
AC = Absorbance of control
AS = Absorbance of samples

The result of each sample was an average of triplicate (Pavithra & Vadivukkarasi, 2015).

**Mineral analysis:** In the environmental science lab, the minerals analysis *of P. hydropiper* was done at the Agriculture University of Khyber Pakhtunkhwa, Pakistan. Adhering to standard protocol *P. hydropiper* was examined by wet digestion methods.

The two grams of sample powder were taken in a 1000 mL flask for the digestion of the plant. In the digestion flask, different solutions of $HNO_3$, $H_2SO_4$, and HCL were added with a ratio of 20:8:2. The sample and the solution of acids ($HNO_3$, HCL, and $H_2SO_4$) were poured into the digestion flask and placed on a hot plate at fume hood. The Hot plate temperature was kept at 200°C and was gradually increased up to 380°C for a time of 1 hour. The volume of the solution or mixture was reduced up to 3 mL and black crystals were obtained. Then the flask was placed in a Fume hood and allowed to cool, after that the addition of 100 mL distilled water was taken place in a flask and poured in a blackish color. The solution was mixed thoroughly and was filtered using a filter paper 3-4 times till a pure white crystalline mixture was obtained. The distilled water was added to 100 mL which was used for the atomic absorption spectrophotometer**.**

**MS analysis:** The GC-MS (Gas Chromatography-Mass Spectrometry) analysis, conducted at Comsat University Abbottabad, employed a rigorous protocol for the isolation and characterization of volatile and semi-volatile compounds present in the samples. This process involved several key steps to ensure accurate and reliable results. Initially, the samples were meticulously prepared, involving the removal of non-volatile components and the concentration of volatile and semi-volatile compounds to enhance detectability. Subsequently, the GC-MS instrument was meticulously set up, with a crucial element being the chromatographic column inside the analyzer. Notably, the temperature range was a critical parameter in the analysis, spanning from 40ºC to 240ºC. This controlled temperature range facilitated the separation of compounds based on their boiling points, with lower boiling point compounds eluting at lower temperatures and vice versa. Helium gas was chosen as the carrier gas, which played a pivotal role in transporting the sample through the chromatographic column for separation. In essence, this method ensured the precise isolation and identification of the target compounds, making it a fundamental tool in chemical analysis and research (Ali *at al*., 2019).

## Results

**Phytochemical analysis:** The present study revealed that *P. hydropiper* extracts are subjected to sufficient chemical tests to confirm the existence of phytochemicals such as tannin, saponins, flavonoids, terpenoids, alkaloids, glycosides, volatile, phenols, steroids carotenoids and proteins. In the *P. hydropiper*, the alkaloids were present in the highest amount in D. water, n-hexane, and ethyl acetate extracts and moderately present in chloroform and methanolic extracts. The tannins were moderately present in ethyl acetate and partially present in aqueous and methanol. Saponins were only partially present in chloroform and methanol extracts. While were not present in D. water, n-hexane, and ethyl acetate extracts. Flavonoids were moderately present in Aqueous while not present in all other extracts. Terpenoids were moderately present in chloroform and methanol extracts and partially present in ethyl acetate extracts while were not present in aqueous and n-hexane extracts. the glycosides were

moderately present in methanol and partially present in ethyl acetate and Aqueous extracts. Volatile oils were partially present in all extracts except in n-hexane extracts. Phenols were moderately present in ethyl acetate and partially present in aqueous and methanol's While were not present in chloroform and n-hexane. The steroids were partially present in chloroform and methanol extracts while were not present in D. water, n-hexane, and ethyl acetate. The carotenoids were moderately present in Aqueous and methanol extracts (Table 1).

**Antibacterial activities:** The antibacterial activity of *P. hydropiper* was tested against 5 different bacterial strains using the disc diffusion method. The highest zone of inhibition was 24 ± 0.72 and 23 ± 0.31 mm in ethyl acetate and chloroform extracts while in the *Klebsiella* species the zone of inhibition was against distilled water and chloroform extracts (Table 2).

**Antifungal activity:** The Antifungal activity of *P. hydropiper* extracts was tested against four fungal strains i.e., *Aspergillus flavus*, *Fusarium solani*, *Aspergillus fumigatus*, and *Mucor* Species. The most efficient and good results were shown in all five extracts against *Aspergillus flavus* and *mucor* species (Fig. 1).

**Anti-nematodal activities:** Various partitions phases of *P. hydropiper* have shown the maximum death ratio at minimum concentration. Among all, D. water extract showed the maximum activity followed by the chloroform extract and ethyl acetate extracts while methanol and n-hexane extracts showed the minimum death ratio of c-elegans (Fig. 2).

**Fluorescence microscopy:** The fluorescence microscopy of *C. elegans* showed good results in the chloroform extracts. The internal structure i.e., the digestive tract (elementary canal) was completely damaged by chloroformed extract (Fig. 3).

***In vitro* anti-diabetics activity:** The maximum enzyme inhibitory activity of the *P. hydropiper* was 50.81% in n-hexane and ethyl acetate extracts followed by aqueous extract (30.32%) while methanolic and chloroform extracts were 26.30% and 21.51% respectively. These results were 70.36% in *P. hydropiper* in aqueous extracts (Fig. 4).

***In vivo* antidiabetic activity of *P. hydropiper*:** The alloxan-induced mice were used for the determination of anti-diabetic potential and the determined data were expressed in Table 3. It was expressed that in the results that major rise in diabetic control was reported at a range of 1st day to 10$^{th}$ days. The results of *P. hydropiper* extracts were shown from zero first day up to Ten days. The changes of blood glucose level in diabetic control at different concentrations of plant extracts and standard glibenclamide. It was observed that the glucose level of the diabetic control was significantly decreased with respect to time. Initially, the oral administration of plant extracts (100mg/kg) was checked and observed the considerable 70% reduction in glucose level of diabetic animals in various extracts.

**Table 1. Phytochemicals present in different extracts of *P. hydropiper*.**

| Phytochemicals | Chloroform | D. Water | N. Hexane | Methanol | Ethyl Acetate |
|---|---|---|---|---|---|
| Tannin | - | + | - | + | ++ |
| Saponins | + | - | - | + | - |
| Flavonoids | - | ++ | - | - | - |
| Terpenoids | ++ | - | - | ++ | + |
| Alkaloids | ++ | +++ | +++ | ++ | +++ |
| Glycosides | - | + | - | ++ | + |
| Volatile oil | + | + | - | + | + |
| Phenol | - | + | - | + | ++ |
| Steroids | + | - | - | + | - |
| Carotenoids | + | ++ | ++ | ++ | + |

Here, +++ = Present in large quantity, + = Low quantity and - = Not detected

**Table 2. Antibacterial activities of various fractions of *Persicaria hydropiper*.**

| Extracts | *E. coli* | MIC (100µg/mL) | k. p | MIC | B. S | MIC (100µg/mL) | S. p | MIC (100µg/mL) | S. A | MIC (100µg/mL) |
|---|---|---|---|---|---|---|---|---|---|---|
| Ethyl acetate | 24±0.72$^{a}$ | 6±0.61$^{c}$ | 6±0.45$^{b}$ | NIL | 7±.087$^{b}$ | NIL | 9±0.87$^{b}$ | NIL | 11±0.77$^{b}$ | NIL |
| N-Hexane | 19±3.37$^{b}$ | 9±0.89$^{a}$ | 9±0.23$^{b}$ | NIL | 9±0.87$^{b}$ | NIL | 9±0.63$^{b}$ | NIL | 10±0.29$^{b}$ | NIL |
| Methanol | 16±0.34$^{c}$ | 8±0.34$^{b}$ | 8±0.71$^{b}$ | NIL | 6±0.35$^{b}$ | NIL | 7±0.37$^{b}$ | NIL | 6±0.76$^{b}$ | NIL |
| D.Water | 18±0.41$^{c}$ | 7±0.31$^{b}$ | 11±0.98$^{b}$ | NIL | 7±0.45$^{b}$ | NIL | 8±0.53$^{b}$ | NIL | 9±0.71$^{b}$ | NIL |
| Chloroform | 23±0.31$^{a}$ | 10±0.56$^{a}$ | 10±0.66$^{b}$ | NIL | 9±0.45$^{b}$ | NIL | 9±0.76$^{b}$ | NIL | 8±0.75$^{b}$ | NIL |
| Positive | 20±0.84$^{b}$ | 1.11$^{d}$ | 16±0.82$^{a}$ | 3.33 | 30±0.11$^{a}$ | 0.37 | 22±0.05$^{a}$ | 1.11 | 25±0.07$^{a}$ | 0.37 |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Here, K.P (*Klebsiella pneumonia*), B.S (*Bacillus subtilis*), S.P (*Streptococcus pneumonia*) and S.A stands for, *Staphylococcus aureus*

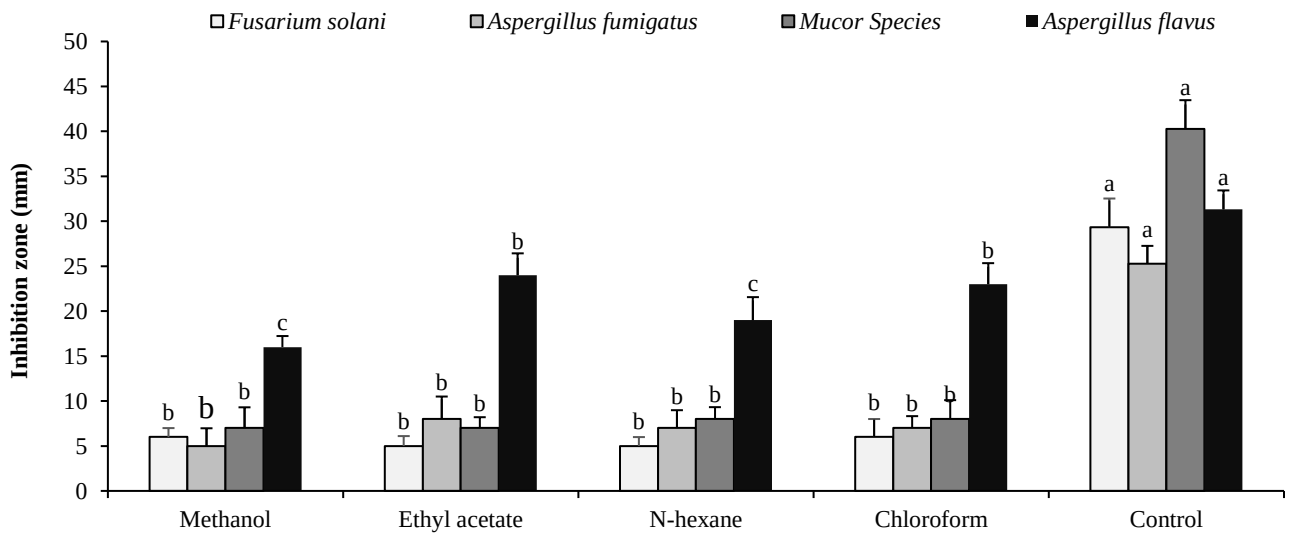


Fig. 1. Antifungal activity of different partition phases of *P. hydropiper* extracts tested against fungal strains. Various bars showed Means ± SE of triplicates. Similarly, different statistical letters represent significant statistical differences from their respective control at $p \leq 0.05$.

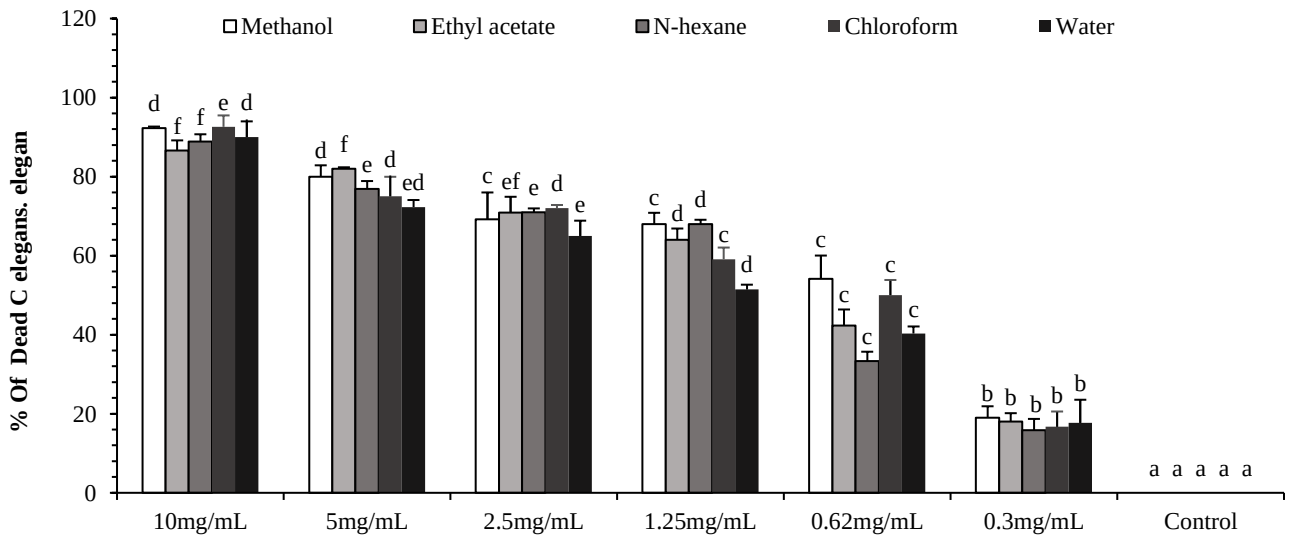


Fig. 2. Antinematodal activity of different partition phases of *P. hydropiper*. Various bars showed Means ± SE of triplicates. Similarly, different statistical letters represent significant statistical differences from their respective control at $p \leq 0.05$.

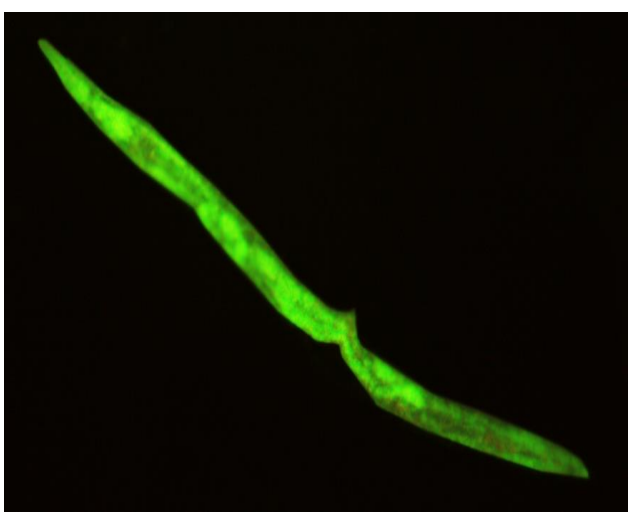

Fig. 3. Fluorescence microscopy of antinematicidal activity of *P. hydropiper*.

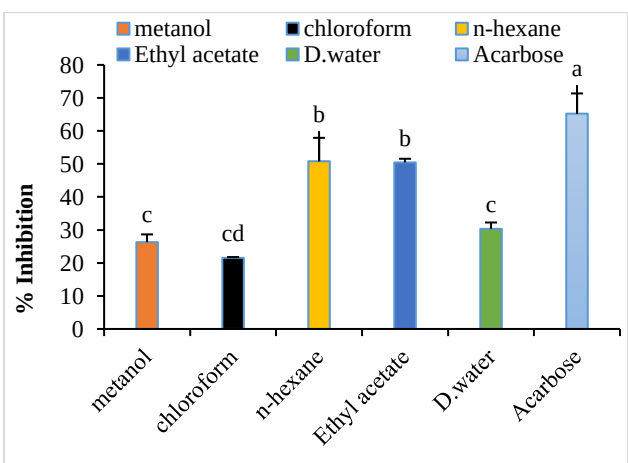


Fig. 4. *In vitro* antidiabetics activity of different partition phases of *P. hydropiper*. Various bars showed Means ± SE of triplicates. Similarly, different statistical letters represent significant statistical differences from their respective control at $p \leq 0.05$.

**Table 3. Antidiabetic activity of *P. hydropiper.***

| Groups | Dose mg/kg | Day 1st | Day 3rd | Day 5th | Day 10th |
|---|---|---|---|---|---|
| Normal control | 0.5mL | 130.4 ± 3.33$^{d}$ | 142.9 ± 7.94$^{d}$ | 159.0 ± 3.66$^{e}$ | 140.0 ± 4.31$^{c}$ |
| Diabetic control | 0.5mL (150mg/kg) | 250. 4 ± 1.23$^{cd}$ | 288.4 ± 6.73$^{b}$ | 310.2 ± 4.34$^{b}$ | 360.2 ± 3.76$^{a}$ |
| Glibenclamide | 0.5mL (10mg/kg) | 280.5 ± 1.43$^{c}$ | 170.23 ± 1.71$^{d}$ | 140.8 ± 2.15$^{e}$ | 130.8 ± 3.45$^{c}$ |
| Methanolic extract | 100 | 330.16 ± 5.99$^{ab}$ | 300.7 ± 4.55$^{b}$ | 280.45 ± 4.55$^{c}$ | 262 ± 4.70$^{b}$ |
| Chloroform | 100 | 260.37 ± 3.56$^{c}$ | 230.56 ± 4.56$^{c}$ | 200.34 ± 5.56$^{d}$ | 180.89 ± 4.78$^{c}$ |
| N-hexane | 100 | 310.67 ± 4.67$^{b}$ | 290.78 ± 5.56$^{b}$ | 265.89 ± 4.57$^{c}$ | 230.89 ± 5.67$^{b}$ |
| Ethyl acetate | 100 | 303.39 ± 5.88$^{b}$ | 280.63 ± 5.55$^{b}$ | 230.65 ± 6.89$^{c}$ | 190.89 ± 3.77$^{c}$ |
| Aqueous | 100 | 360.44 ± 5.66$^{a}$ | 350.77 ± 7.13$^{a}$ | 330.21 ± 5.99$^{a}$ | 290.98 ± 3.11$^{b}$ |

**Antioxidant activity *Persicaria hydropiper* using DPPH free radicals:** The antioxidant activity of *P. hydropiper* plant extract was determined by the DPPH radical scavenging assay. The current analysis of this plant showed very good antioxidant activities. The three different concentrations were selected for this study i.e., 60ppm, 80ppm, and 100ppm respectively. The Methanolic extracts showed highly efficient antioxidant activity which is 80.25±2.30%.

At 100 ppm interesting anti-oxidant activity was observed in the different extracts i.e., distilled water, ethyl acetate, chloroform, that is (80.25±2.30%), (86.35±2.95%), (81.25±2.30%) respectively which was compared with standard ascorbic acid (98.33±1.23). However, at 80 ppm also considerable effect i.e., n-hexane (81.25 ± 1.98), ethyl acetate extract (81.25 ± 98%), chloroform extract (86.35±2.95%), then *Persicaria hydropiper* at 100 ppm as compared with standard ascorbic acid (98.33 ± 1.28) at 100 ppm.

The antioxidant activity of the *P. hydropiper* at 80 ppm was showed in methanol (76.32 ± 2.42) followed through n-hexane (63.00 ± 1.91), Distilled water (65.67±1.99), ethyl acetate (69.9 ± 1.98), chloroform (77.00 ± 2.77) as compared with standard ascorbic acid 89.97 ± 1.87 at same ppm.

The antioxidant activity of *P. hydropiper* at 60 ppm in methanolic extract (49.35 ± 2.13), followed by n-hexane extract (49.23 ± 1.98), distilled water (52.15 ± 2.15), ethyl acetate (52.20 ± 2.34), chloroform (57.99 ± 1.99) as compared to ascorbic acid (77.29 ± 1.69) as a control was used however, no abnormal and irregular trends were showed in analysis of *Persicaria hydropiper* at 100ppm, 80 ppm, and 60 ppm concentration.

**Mineral analysis:** The different minerals present in *Persicaria hydropiper* are sodium, cobalt, chromium, cobalt, copper, iron lead, manganese, magnesium, nickel, zink, cadmium, and calcium. The detected value of sodium in *P. hydropiper* was 30.00 ± 2.21 mg/100g which was the highest mineral analytical value. On the same way, In *P. hydropiper* copper level observed was 0.217 ± 0.023 mg/100g and thecalculated value of zinc in *P. hydropiper* was 0.125 ± 0.015 mg/100g. The levels of lead in *P. hydropiper* were 0.040 ± 0.005 mg/100g. However, the chromium level observed in *P. hydropiper* was 0.058 ± 0.003 mg/100g. The mineral analytical value of potassium in *P. hydropiper* was 70.00 ± 0.205 mg/100g. The level of calcium in *P. hydropiper* was 25.353 ± 1.24 mg/100g. The magnesium mineral analytical value in *P. hydropiper* was 3.038 ± 0.77 mg/100g. The cobalt value in *P. hydropiper* was 0.010 ± 0.004 mg/100g, the lowest mineral analytical value (Table 5).

**Table 4. Presented results of *P. hydropiper* belonging to the family Polygonaceae showed highly efficient antioxidant potential at 100 ppm in methanolic extracts (82.25 ± 1.93).**

| Extracts | 100 PPM | 80 PPM | 60 PPM |
|---|---|---|---|
| Methanol | 82.25 ± 1.93$^{b}$ | 76.32 ± 2.42$^{b}$ | 49.35 ± 2.13$^{b}$ |
| N-Hexane | 82.00 ± 3.15$^{b}$ | 63.00 ± 1.91$^{c}$ | 49.23 ± 1.98$^{b}$ |
| D. Water | 80.25 ± 2.30$^{b}$ | 65.67 ± 1.99$^{c}$ | 52.15 ± 2.04$^{b}$ |
| Ethyl acetate | 87.00 ± 1.39$^{b}$ | 69.90 ± 1.98$^{b}$ | 52.20 ± 2.34$^{b}$ |
| Chloroform | 96.65 ± 2.23$^{a}$ | 77.2 ± 2.77$^{b}$ | 57.99 ± 1.99$^{b}$ |
| Control | 98.33 ± 1.28a | 89.97 ± 1.87a | 77.29 ± 1.69a |

**Table 5. Mineral analysis of *Persicaria hydropiper.***

| Minerals | (mg/100g) | Minerals | (mg/100g) |
|---|---|---|---|
| Copper | 0.217 ± 0.014 | Chromium | 0.058 ± 0.005 |
| Zinc | 0.125 ± 0.015 | Cobalt | 0.010 ± 0.002 |
| Manganese | 0.025 ± 0.023 | Sodium | 30.00 ± 1.21 |
| Iron | 1.251 ± 0.023 | Potassium | 70.00 ± 0.205 |
| Nickel | 4.753 ± 0.031 | Calcium | 25.353 ± 2.24 |
| Cadmium | 0.047 ± 0.041 | Magnesium | 3.038 ± 0.97 |
| Lead | 0.040 ± 0.006 | | |

**GC-MS analysis of *P. hydropiper*:** GC-MS analysis revealed the presence of 61 compounds among which Benzene Cyclo Hexyle, Sulfurus Acid, 2 Ethyl Hexyledecyle Ester, 2,4-Di-Tert-Butyl Phenol, Cyclobutanone, 2, 3, 3, 4-Tetra Methyl and Pentaoic Acid, 2-Ethyl Hexyl Ester were the most abundant compounds (Fig. 5 and Table 6).

## Discussion

The Phytochemical analysis of *Persicaria hydropiper* showed the maximum results in the n-hexane, ethyl acetate, D. water, methanol, and chloroform extracts. The antibacterial activity of *P. hydropiper* was tested against 5 different bacterial strains using a well-diffusion method. The highest zone of inhibition was 24 ± 0.72 and 23 ± 0.31 mm in ethyl acetate and chloroform extracts while in the klebsiella species the zone of inhibition against distilled water and chloroform extracts. (Aziman *et al*., 2021) recorded the antibacterial activity of *P. hydropiper* in ethanolic extracts while in the study the antibacterial activity of five different extracts i.e., methanol, ethyl acetate, chloroform, n-hexane, and aqueous extracts i.e., very considerable.

The antifungal activity of *P. hydropiper* extracts was tested against four fungal strains i.e., *Aspergillus flavus*, *Fusarium solani*, *Aspergillus fumigatus* and *Mucor* Species. All five extracts against *Aspergillus flavus* and mucor species showed the most efficient and good results. The tissue and body's defense system were protected by antioxidants from free radicals. The present results of *Persicaria hydropiper* belonging to the family Polygonaceae showed highly efficient antioxidant potential at 100 ppm in methanolic extracts (82.25 ± 1.93). According to Aziman, 2021 both ethanolic extract and essential oil of *P. hydropiper* possessed antibacterial activity against *Staphylococcus aureus* only at different concentrations, with mini- mum inhibitory concentration values: 0.625 and 5 mg/mL, respectively; and mini- mum bactericidal concentration values: 5 and 40 mg/mL, respectively. Our results of antioxidant activities showed that these are the highly active and efficient results of *P. hydropiper* plants. The different plant extracts have shown the maximum death ratio at minimum concentration D. water extract showed the maximum activity followed by the chloroform extract and ethyl acetate extracts while methanol and n-hexane extracts showed the minimum death ratio of *C -elegans* (Fatima *et al*., 2015; Meriga *et al*., 2012; Xiang *et al*., 2023).

Hyperglycemia is a metabolic disease characterized by diabetes mellitus, abnormal protein, and lipid metabolism along with specific long-term problems affecting the retina, nervous system, and kidney (Rahman *et al*., 2023). Hyperglycemia is a significant aspect in the development and evolution of the problem of diabetes mellitus. Type II diabetes (non-insulin-dependent diabetes mellitus) is the most prevalent form of diabetes caused by reduced secretion of insulin causing high postprandial glucose levels. One of the most essential problems which increased postprandial glucose concentration is the fast digestion of glucose in the intestine by the action of α- amylase and α-glycosidase that helps in the breakdown of complex carbohydrates into simple sugars such as maltose and glucose (Begum *et al*., 2023). For the treatment of diabetes the inhibition of α- amylase and α-glucosidase were used which involves the decrease postprandial hyperglycemia by causing retardation in the absorption of glucose (Thiviya *et al*., 2022). Hypolipidemia is mostly rare but secondary causes are relatively usual compared to the uncommon primary hypolipidemic conditions. The rate of hypolipidemia depends on plasma cholesterol level which is used to explain the condition. Hypocholesterolemia is found to be associated with several mechanisms that have been defined in different disorders and clinical situations (Tanaka *et al*., 2021).

The powerful inhibiter of lipolysis is the insulin hormone. In diabetes the insulin is deficient, and the lipolysis of lipids is increased due to the action of lipase enzyme and the release of more free fatty acids in the blood (Thiviya *et al*., 2022). When the free fatty acid concentration is increased in blood it in turn increases the beta-oxidation of fatty acids by increasing the action of HMG-CoA reductase for formation more cholesterol. Insulin also increases the receptor-mediated elimination of LDL-cholesterol but in diabetes decreased the activity of insulin and causes hypercholesterolemia (Eddouks *et al*., 2005).

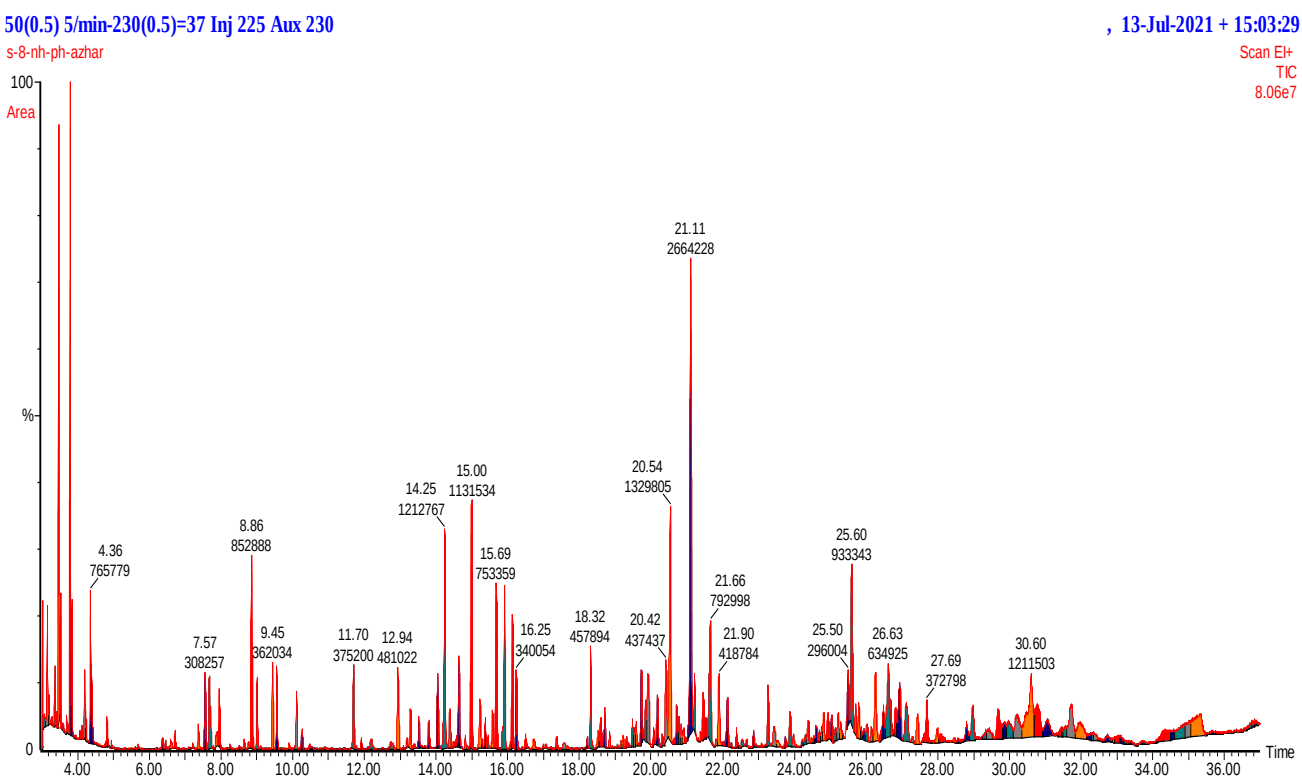


Fig. 5. GC- MS analysis of n-hexane extracts of *P. hydropiper.*

**Table 6. The GC-MS analysis of *P. hydropiper*.**

| S. No. | C-Name | RT (minutes) | Area (%) |
|---|---|---|---|
| 1. | Pentanoic Acid,2-Ethyl Hexyle Ester | 3.48 | 4.93 |
| 2. | 1, Octyle,2-Butyle- | 3.52 | 0.98 |
| 3. | Cyclobutanone,2,3,3,4-Tetra Methyle- | 3.8 | 5.66 |
| 4. | P-Xylene | 4.36 | 2.62 |
| 5. | Benzene,1,3dimethel- | 4.82 | 0.41 |
| 6. | Sulfurus Acid, 2 Ethyl Hexyledecyle Ester | 7.57 | 10.74 |
| 7. | 2,6,10-Trimethyle Tridecane | 8.86 | 1.51 |
| 8. | 1r,2c,3t,4t-Tetramethyl-Cyclohexane | 9.46 | 1.25 |
| 9. | Benzene Cyclo Hexyle | 11.7 | 13.04 |
| 11. | Cyclohexane Carboxylic Acid 1, (1,1-Dimethyle)- | 12.51 | 0.01 |
| 12. | Butenyle Tiglate,3-Methyle-3- | 12.59 | 0.002 |
| 14. | Ethoxy Cyclohexyledimethyl Silane | 12.74 | 0.10 |
| 15. | Sulfurous Acid,2ethyle Hexyle Tridecyle Ester | 12.8 | 0.10 |
| 16. | Heptadecane | 12..94 | 1.67 |
| 20. | Carbonic Acid, 2-Ethylehexyle Undecyle Ester | 13.29 | 0.61 |
| 22. | Cyclononane,1,1,4,4,7,7-Hexamethyle | 13.53 | 0.46 |
| 23. | Heneiocosane | 14.05 | 1.22 |
| 24. | Benzene,1,3-Bis (1,1-Dimethyle Ethyle) | 14.25 | 4.21 |
| 25. | Hentriacontane | 14.39 | 0.67 |
| 26. | Octane 3,3-Dimethyl- | 14.49 | 0.08 |
| 27. | Sulfurous Acid,2-Ethylhexyltridecyl Ester | 14.58 | 0.19 |
| 28. | Octadecane,2,6,10,14-Tetramethyl- | 14.65 | 1.43 |
| 29. | Octacosane,1-Iodo- | 14.76 | 0.01 |
| 30. | Dodecane,1, Flouro | 14.87 | 0.04 |
| 31. | Squalane | 15 | 3.93 |
| 32. | Triacontane | 15.58 | 0.50 |
| 33. | 1-Decanol,2hexyl- | 15.69 | 2.61 |
| 34. | Beta-D-Lyxofuranoside, Thio-Decyle- | 15.19 | 0.005 |
| 35. | 1r,2c,3t,4t-Tetramethyl-Cyclohexane | 15.92 | 3.15 |
| 37. | Hexadecane | 16.08 | 0.10 |
| 38. | 8-Heptadecane | 16.14 | 1.97 |
| 39. | 0ctadecane,2,6,10,14-Tetramethyl- | 16.25 | 1.18 |
| 40. | Heptane,2,2,3,3,5,6,6-Heptamethyl- | 16.41 | 0.03 |
| 41. | Dodecane2,6,10-Trimethyl- | 16.5 | 0.29 |
| 42. | Decane,3,8, Dimethyl- | 18.32 | 1.59 |
| 43. | Hentriacontane | 18.53 | 0.33 |
| 44. | Triacontane | 18.61 | 0.52 |
| 45. | 2-Methylhentriacontane | 18.71 | 0.62 |
| 46. | Tritetracontane | 18.85 | 0.23 |
| 47. | Nonadecane,2,6,10,14,18-Pentamethyl- | 19.92 | 1.72 |
| 48. | Dotriacontane | 20.54 | 4.62 |
| 51. | 2,4-Di-Tert-Butyl Phenol | 21.11 | 9.26 |
| 52. | Pentacosane | 23.88 | 0.70 |
| 53. | Heneicosane | 24.38 | 0.50 |
| 54. | 0ctacosane,2-Methyl- | 24.82 | 0.57 |
| 55. | 6-Tetradecanesulponicacid, Butyl Ester | 25.6 | 3.24 |
| 56. | Dotriacontyl Pentafluoropropionate | 26.26 | 1.73 |
| 57. | Tetracontane | 26.63 | 2.20 |
| 58. | 1-Hexacosanol | 27.69 | 1.29 |
| 59. | Bis (Tridecyl)Phthalate | 28.98 | 1.09 |
| 60. | Orsine, Oxophenyl- | 29.67 | 1.35 |
| 61. | Hexacontane | 30.6 | 2.48 |

Hypoglycemic properties have been reported in many traditional medicinal plants such as *Allium sativum* (Garlic), *Trigonella frenum* (Fenugreek), *Ocimum santum* (Tulsi) *Azadirachta indica* (Neem), *Vinca rosea* (Nayantara), *Momordica charantia* (Bitter ground) and *Carraluma fimbriata* (Choung). Several of these plants are less effective in decreasing blood glucose concentration in serious diabetes (van de Venter *et al*., 2008). In the *Pithecellobium dulce* leaves the insulin-like principle has been reported. Traditionally the seeds powder of Pithecellobium dulce is mixed with the tender leaf paste and is given orally on an empty stomach to cure diabetes (Rizvi *et al*., 2013). The following plants show the hypolipidemic activity; Grain amaranth, Inca wheat, Palash, Amaltas, Guggul, Kesuti, Kesraj, Jam, Kalojam, Kalajam, Pipal, Mulethi (Pankaj *et al*., 2015). It was previously reported that the fruit of *P. embilica* has a necessary component of several Ayurvedic multi-herbal preparations which are still commonly used to treat many diseases including diarrhea, jaundice, inflammation, cerebral and intestinal disorders, diabetes mellitus, coronary heart disease, cancer, and rheumatic pain, diseases of the eye and genitalia, gonorrhea, constipation, asthma, biliousness and as a tonic for hair (Rashidi *et al*., 2016).

A total of 61 compounds were isolated and identified from the GC-MS analysis of n-hexane extracts of *P. hydropiper* exhibiting various phytochemical activities. The chromatogram was presented in Figure 4, while the chemical constituents with their retention time (RT), Molecular mass (M. mass), and area concentration (%) are presented in Table 6. The following compounds in Figure 12, were present in the GC-MS analysis carried out on n-hexane extract of *P. hydropiper*. The five maximum concentrations were analyzed in the chromatogram observed in area % shown in Table 6. The five most abundant compounds were Benzene Cyclo Hexyle (RT 11.7), % area 13.04921277, Sulfurus Acid, 2 Ethyl Hexyledecyle Ester, (RT 7.57), % area 10.74018514, 2,4-Di-Tert-Butyl Phenol (RT 21.11), area % 9.265518372, Cyclobutanone, 2, 3, 3, 4-Tetra Methyle- (RT 3.8), % area 5.669511176, and Pentaoic Acid,2-Ethyl Hexyle Ester (RT 3.48), % area 4.933723653.

Mahnashi *et al.*, also recorded the GC-MS analysis of the 141 compounds having very small peak sizes also but, in this research, all the five extracts were analyzed which were considerable (Mahnashi *et al*., 2022). In the previous study, ethanol as a polar organic solvent was used due to its wide solubility selection. Furthermore, from a toxicological point of view, this solvent is much safer and cheaper than other organic solvents such as methanol and acetone (Ali *et al*., 2017; Gan *et al*., 2023). Dried *P. hydropiper* were extracted using 70% ethanol extraction method to obtain the crude extract, and hydrodistillation method to obtain the essential oil. The percentage yield of the ethanolic extract and essential oil of *P. hydropiper*. *The P. hydropiper* ethanolic extract had yield of 11.02%. The *P. hydropiper* essential oil had yield of 0.70%. A low extraction yield of ethanolic *P. hydropiper* extract (1.47%) was reported by Lee and Vairappan (2011). According to Rusdi *et al*., (2016), Polygonum minus (family: Polygonaceae) yellowish essential oil was obtained at 0.475% yield Rusdi *et al*., 2016).

## Conclusion

Based on the various biological assays, phytochemical analyses, and mineral analyses conducted on *P. hydropiper*, it is evident that this plant holds significant potential for traditional medicinal purposes. The diverse range of phytochemicals and biological profiles identified in *P. hydropiper* underscores its medicinal value. Moreover, the successful extraction of these phytochemicals using various solvent systems indicates the versatility of this plant for medicinal applications. The findings of this study present valuable insights into the phytochemical composition of *P. hydropiper*, which could be harnessed for the prevention and treatment of various fetal diseases. The wealth of information generated through these analyses paves the way for further research and development in the field of traditional medicine, with *P. hydropiper* as a promising candidate for future medicinal formulations. However, the comprehensive analysis of *P. hydropiper* conducted in this study highlights its potential as a valuable resource in traditional medicine, particularly for addressing fetal diseases. Further investigations and clinical studies are essential to fully unlock the therapeutic potential of this plant and translate it into practical healthcare solutions.

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