https://doi.org/ 10.48047/AFJBS.6.7.2024.1884-1898

Assessment of Phytochemical, Antimicrobial, Antioxidant activities and HPLC analysis of stem, leaves and fruits of plant *Pyrus pashia*

Kanupriya Kuniyal[1], Niki Nautiyal[1], Geeta Bhandari[2]

[1]Department of Biochemistry and Biotechnology, Sardar Bhagwan Singh University Balawala, Dehradun-248001, Uttarakhand, India
[2] Himalayan School of Biosciences, Swami Rama Himalayan University, Jolly Grant, Dehradun, Uttarakhand, 248140

Volume 6,Issue 7, 2024
Received: 29 Mar 2024
Accepted : 22 May 2024
doi: 10.48047/AF5BS.6.7. 2024. 1884-1898

Abstract

People have long understood eating wild edible fruits has nutritional and therapeutic benefits. One of these lesser-known gems is *Pyrus pashia*, the Himalayan Pear or Wild Edible. This tree, which can grow up to 2,000 meters in elevation in the Western Himalayas and other temperate zones, is largely unutilized. Its many medicinal benefits have long been recognized by traditional medicine, which uses it to treat a wide range of conditions including eye infections, sore throats, diarrhea, abdomen problems, and many infectious diseases. The present work investigates the phytochemical screening, antibacterial, antifungal properties and antioxidant potential of different plant part extracts of *Pyrus pashia* and HPLC analysis of Ethyl acetate and Ethanol extracts for the analysis of three phenolic acids and three flavonoids. The stem, leaves, and fruits underwent extraction using various polar solvents such as water, ethanol, petroleum ether, chloroform, and ethyl acetate. Phytochemical screening revealed the presence of alkaloids, phenols, tannins, flavonoids, saponins, glycosides, and other compounds. The antimicrobial activity against multiple pathogens, including Enterobacter aerogenes, Klebsiella pneumoniae, E. coli, Penicillium sp., Aspergillus sp., and Rhizopus sp., was evaluated using the agar well diffusion technique. Furthermore, the antioxidant capacity of each extract was determined by assessing its ability to scavenge free radicals (2,2-diphenyl picrylhydrazyl). *Pyrus pashia* exhibited great antimicrobial and antioxidant potential. The maximum property was seen in the extract of Ethyl acetate. HPLC analysis showed the availability of compounds of phenols.

Keywords: Phytochemicals, Secondary metabolites, Antimicrobial, Antifungal, Zone of inhibition, Antioxidant, HPLC.

INTRODUCTION

The Himalayan region stands as a symbol of awe-inspiring snow-capped summits, boasting a rich and diverse array of flora alongside abundant natural wealth. Within this expanse lies a treasure trove of aromatic and medicinal plants, vital to industries spanning pharmaceuticals, fragrances, and traditional medicine [1]. For generations, Indian traditional healers have harnessed the healing potential of these herbs, employing them to treat an extensive spectrum of ailments.

Pyrus pashia, also known as kainth, is a fruiting plant that belongs to the Rosaceae family. This deciduous species thrives in predominantly damp soil and typically attains a modest to medium size. Its blossoms adorn the landscape mainly during February and March, giving way to sweet and delectable fruits upon full ripening. The fruit reaches its peak maturity between May and December. Besides kainth, *Pyrus pashia* is recognized by various common names such as Indian wild pear, Himalayan pear, Mehal, Batangi, Molu, and Tangai [2]. Primarily cultivated in the Himalayan regions of India [3], this plant is esteemed for its nutritional and therapeutic properties yet remains an underutilized species.

Traditional and pharmacological importance of *Pyrus pashia*

P. pashia is widely utilized by many local cultures to treat gastrointestinal, respiratory, and circulatory issues and is spread with enormous ethnic advantages [4]. The ripe fruits of P. pashia have been utilized as a constipation remedy by the local populace. The apple juice contains diuretic and astringent qualities [5]. The fruit extract has historically been used for ailments like eye disorders, digestive issues (dyspepsia), headaches, diaphoretic sweats, abdominal pain and dysmenorrhea [6]. In order to increase the production of milk, the fruits are also fed to animals that produce milk [7]. Cough, emesis, and diarrhea can also be treated with flower decoction [8]. Fresh leaves of P. pashia are recognized for their sedative qualities. Additionally, leaves find application in cosmetics [9].

Historically, the extracts of leaves have served as a tonic for addressing hair issues [10]. In Ayurvedic practice, the bark and roots of P. pashia fruits are employed to effectively treat fever, peptic and stomach ulcers, as well as sore throats [11]. Additionally, a tonic derived from the bark is utilized in the treatment of typhoid fever, boasting astringent, laxative, anthelmintic, and febrifuge properties [12].

The broad pharmacological activities are anti-inflammatory and antiproliferative activity, antioxidant activity, antimicrobial and antifungal activities, gastrointestinal, hepatoprotective activity, spasmolytic, bronchodilator and vasoconstrictive activities, treatment of gastrointestinal disorders, respiratory and cardiovascular ailments, anti-depressant activity, disinfectant and reduce risk of high cholesterol.

(a) **(b)** **(c)**

Fig 1: (a) Tree (b) Flower (c) Fruit of *Pyrus pashia*

MATERIALS AND METHODS

Plant sample collection

Pyrus pashia plant stems and leaves are collected in the month of February 2023 and fruitswere collected in the month of June 2023 from Narayanbagarh, Chamoli, Uttarakhand.

Plant extract preparation

After rinsing off soil particles with distilled water, the plant material underwent a drying process in the shade lasting 15 to 20 days. Once dry, the material was finely ground into a powder using an electric blender and stored. Subsequently, the dried material was subjected to Soxhlation using a series of solvents, with each solvent amounting to 800 ml. The resulting extracts were then refrigerated at 4°C in an airtight container, primed for further research.

Preparation of different concentrations of plant extract

The plant extracts of different solvents were dried, stock solution of 5mg/ml was prepared. The desired concentrations were made by using the formula: C1 * V1 = C2 * V2

C1 = Stock concentration, V1 = Volume of Stock required to prepare new solution, C2 = Concentration of new/working solution, V2 = Volume of new solution desired. The concentrations are made by dissolving dried extracts with their respective solvents.

Qualitative phytochemical analysis

The freshly prepared extracts underwent standard preliminary phytochemical analysis to confirm the availability of the following phytoconstituent includes flavonoids, tannins, compounds of phenol, alkaloids, glycosides, and carbohydrates.

Evaluation of Antibacterial and Antifungal activity

Microorganisms:

The Department of Biochemistry and Biotechnology provided the pure cultures of every bacterium that was examined. The pure cultures were subcultured and kept on Nutrient Broth at 4˚C. Enterobacter aerogenes, Klebsiella pneumoniae, Escherichia coli, Penicillium sp., Aspergillus sp., and Rhizopus sp. are the species that were employed.

Agar Well Diffusion Assay:

The antibacterial and antifungal susceptibility of the plant extracts were evaluated using Mueller Hinton Agar plates and the agar well diffusion technique [13]. Initially, the test microorganisms were cultured on the agar medium surface. The plates were then uniformly inoculated with microorganisms using a sterile cotton swab. Subsequently, five wells, each with a diameter of 6 mm, were created at equal intervals using either a sterile cork-borer or micropipette tips. Twenty microliters of various concentration extracts were added to each well, and the plates were then incubated at 37°C for a full day. After incubation, the plates were examined for clear zones surrounding the wells, indicating antibacterial or antifungal activity of the samples. The zone of inhibition (ZOI) for each sample was measured in millimeters using a scale and recorded [14].

Evaluation Antioxidant activity

DPPH assay for determination of Free Radical Scavenging Activity:

To assess the plant extracts the DPPH (1,1-diphenyl-2-picrylhydrazyl) technique was employed [15]. Ascorbic acid served as the reference standard. Initially, a freshly prepared 0.1 mM DPPH solution (4 mg in 100 ml) was created in methanol. Plant extracts were then prepared at various concentrations using dimethyl sulfoxide (DMSO), ranging up to 40 µL. Test tubes containing 2.96 mL of the freshly prepared DPPH solution were supplemented with the respective extract concentrations. After thorough mixing, the mixtures were allowed to stand for thirty minutes. The degree of radical inhibition was indicated by a color change from purple to yellow. Subsequently, the absorbance was assessed at 517 nm employing a UV-visible spectrophotometer, with methanol utilized as the baseline. Lower absorbance readings from the spectrophotometer suggested a higher capacity to scavenge free radicals [16]. The percentage scavenging activity of DPPH was calculated using the following formula:

$$\% \text{ Radical inhibition} = ([Ac - At]/ Ao) \times 100$$

Where, (Ac) is the absorbance of the control reaction and (At) is the absorbance of the sample of the extracts.

HPLC analysis

HPLC profiles of Ethyl acetate Stem, Ethyl acetate Leaves, Ethyl acetate Fruit and Ethanol fruit extracts of *Pyrus pashia* were determined. Gallic acid, vanillic acid, hydroxybenzoic acid, rutin, catechin and quercetin were used as standard solutions for the quantification of phenolic compounds.

RESULT AND DISCUSSION

Preliminary phytochemical analysis/ Qualitative Phytochemical analysis

Initial phytochemical screening of various extracts from *Pyrus pashia* stem, leaves, and fruits revealed the presence of diverse phytochemical groups, including alkaloids, tannins, saponins, carbohydrates, steroids, glycosides, flavonoids, and terpenes. The results indicated the availability of the following:

Table 1: Results of phytochemical test performed on different extracts

Plant part	Test performed	Petroleum ether	Chloro-form	Ethyl acetate	Ethanol	Water
Stem	Dragendroff's test	+	+	+	+	-
	Alkaline reagent test	-	+	+	+	+
	Lead Acetate Test	+	+	+	+	+
	Froth test	-	+	+	+	+
	Molisch's test	+	+	+	+	+
	Kieller Kiliani test	-	+	+	+	+
Leaves	Dragendroff's test	-	+	+	+	-
	Alkaline reagent test	+	+	+	+	-
	Lead Acetate Test	+	+	+	+	+
	Froth test	-	+	+	+	+
	Molisch's test	+	+	+	+	+
	Kieller Kiliani test	+	+	+	-	+
Fruits	Dragendroff's test	-	+	+	+	-
	Alkaline reagent test	+	+	+	+	-
	Lead Acetate Test	+	+	+	+	-
	Froth test	+	+	+	+	+
	Molisch's test	+	+	+	+	+
	Kieller Kiliani test	-	+	+	+	-

Antibacterial activity

Different extract concentrations ranging from 1 mg/ml to 5 mg/ml exhibited varying widths of zones of inhibition (ZOI), which serve as indicators of the extract's relative antibacterial activity. Among these, extracts containing ethyl acetate demonstrated the highest activity levels, while water extracts exhibited lower activity levels, with the lowest ZOI observed. Petri plates containing the chloroform extract showed either no or very minimal bacterial growth. Remarkably, ethanol and ethyl acetate extracts displayed superior effectiveness, significantly inhibiting the majority of the investigated microorganisms.

Table 2: Zone of inhibition (mm) of various extracts of *Pyrus pashia* (a) Stem (b) Leaves (c) Fruits on different microbial strains

	Zone of Inhibition (mm)														
	Enterobacter aerogenes					*Klebsiella pneumoniae*					*E. coli*				
	Concentration (mg/ml)														
Extracts	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Petroleum ether	10	10	11	11	12	-	-	-	10	11	10	11	12	12	13
Chloroform	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethyl acetate	11	16	18	22	**26**	12	13	14	16	18	12	14	15	16	20
Ethanol	11	12	13	15	16	10	10	11	12	13	12	13	13	14	16
Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10

	Zone of Inhibition (mm)														
	Enterobacter aerogenes					*Klebsiella pneumoniae*					*E. coli*				
	Concentration (mg/ml)														
Extracts	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Petroleum ether	10	11	11	12	12	-	-	10	10	11	10	10	11	12	12
Chloroform	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethyl acetate	16	18	20	20	**22**	13	15	15	16	18	-	-	-	-	-
Ethanol	10	11	11	11	12	10	10	11	11	12	12	13	13	14	16
Water	-	-	-	-	-	-	-	-	-	-	13	14	15	15	16

(a) (b)

	Zone of Inhibition (mm)														
	Enterobacter aerogenes					*Klebsiella pneumoniae*					*E. coli*				
	Concentration (mg/ml)														
Extracts	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Petroleum ether	10	10	11	11	12	-	-	10	10	11	10	11	12	12	13
Chloroform	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethyl acetate	20	22	22	23	**23**	12	13	16	18	20	12	14	16	17	18
Ethanol	10	11	12	14	16	10	10	11	13	15	12	13	13	14	16
Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(c)

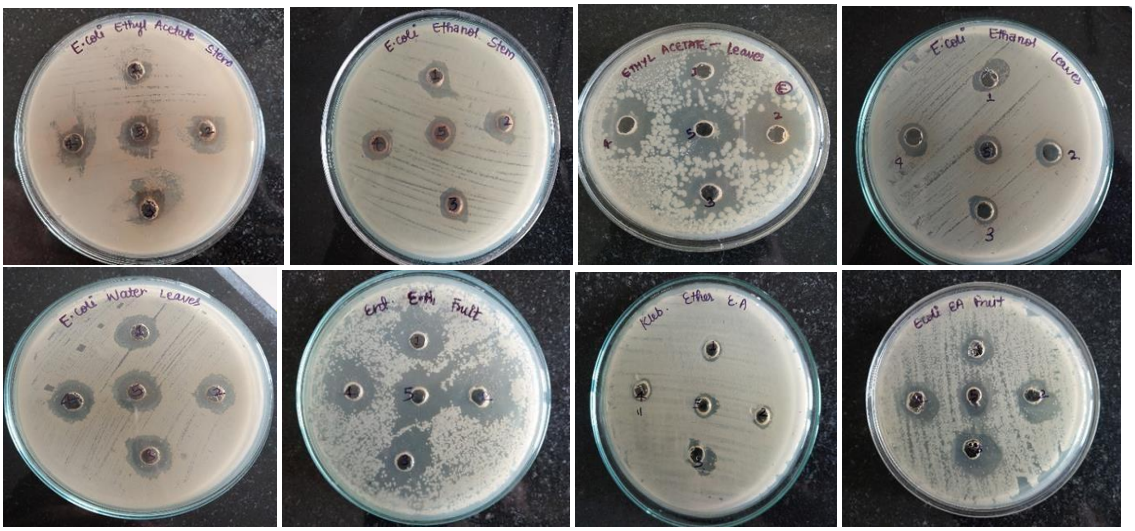


Fig 2: Representation of zone of inhibition of various extracts of *Pyrus pashia* Stem, Leaves and Fruits on different microbial strains

Antifungal properties

The diameter of the zone of inhibition (ZOI) serves as an indicator of the relative antifungal activity of the extract. It was observed that the extracts obtained through ethyl acetate exhibited the highest levels of activity. The extractsof water showed lower activities. No or less fungal growth was seen in the Chloroform extract petri plates. Ethanol and Ethyl acetate extracts were found to be more potent, being capable of exerting significant

inhibitory activities against most of the fungus investigated.

Table 3: Zone of inhibition (mm) of various extracts of *Pyrus pashia* (a)Stem (b) Leaves (c) Fruits on different fungal strains

	Zone of Inhibition (mm)														
	Penicillium sp.					*Aspergillus* sp.					*Rhizopus* sp.				
	Concentration (mg/ml)														
Extracts	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Petroleum ether	-	-	-	-	-	11	12	13	14	15	-	-	12	12	13
Chloroform	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethyl acetate	12	15	16	19	**20**	10	12	13	13	15	11	12	14	17	19
Ethanol	-	-	-	-	-	12	14	16	16	17	12	13	14	15	**20**
Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(a)

	Zone of Inhibition (mm)														
	Penicillium sp.					*Aspergillus* sp.					*Rhizopus* sp.				
	Concentration (mg/ml)														
Extracts	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Petroleum ether	-	-	-	-	-	11	12	13	15	18	-	10	12	12	13
Chloroform	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethyl acetate	11	15	16	18	20	18	20	24	28	**30**	16	20	22	25	28
Ethanol	-	-	-	-	-	10	10	11	12	13	-	10	12	13	14
Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(b)

	Zone of Inhibition (mm)														
	Penicillium sp.					*Aspergillus* sp.					*Rhizopus* sp.				
	Concentration (mg/ml)														
Extracts	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Petroleum ether	-	-	-	-	-	11	12	13	14	15	10	10	12	12	14
Chloroform	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethyl acetate	10	11	11	12	14	24	26	30	32	36	12	13	14	15	16
Ethanol	-	-	10	11	12	10	10	11	12	13	10	11	12	13	16
Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

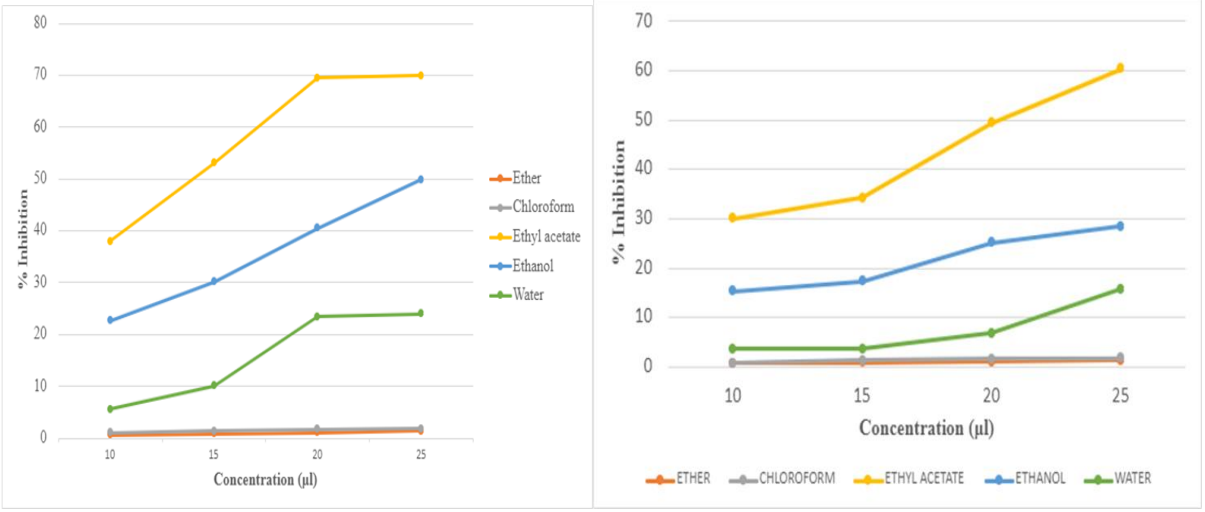
(c)

Fig 3: Representation of zone of inhibition of various extracts of *Pyrus pashia* Stem, Leaves and Fruits on different microbial strains.

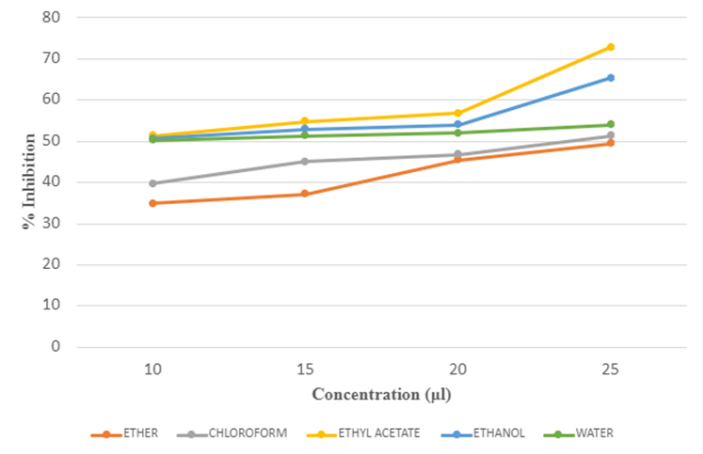
ANTIOXIDANT PROPERTIES

DPPH Scavenging Activity

Figure 4 shows the scavenging effects of samples on DPPH radical for the different concentrations (10 µl, 15 µl, 20 µl and 25 µl). Maximum scavenging activity was shown by Ethyl acetate extract while Petroleum ether had the lowest activity. The study revealed that extracts from the Pyrus pashia plant possess notable antioxidant activity, albeit lower than that of ascorbic acid. This difference could be attributed to the predominant presence of phenolic compounds in these fractions, particularly phenolic hydroxyls, which likely contribute to their observed high levels of antiradical activity.



(a) (b)



(c)

Fig 4: Antioxidant activities of different extracts of *Pyrus pashia* by different solvents at various concentrations a) Stem b) Leaves c) Fruits

HPLC ANALYSIS FOR PHENOLIC COMPOUNDS

For the HPLC analysis, a mixture of 6 phenolic compounds standards (3 phenolic acids, and 3 flavonoids) was injected into the HPLC system. Four samples of plant extract (Ethyl acetate stem, Ethyl acetate leaves, Ethyl acetate fruit and Ethanol fruit) were analyzed. The wavelength taken of standards phenolic acids (gallic acid, vanillic acid, hydroxybenzoic acid) and flavonoids (rutin, catechin and quercetin) are shown in Table: 4. All the extracts show thecharacteristics peak of vanillic acid.

Table 4: Wavelength of standard phenolic compounds

Standard	Wavelength (nm)
Gallic acid	272
Catechin	272
Rutin	272
Quercetin	272
Vanillic acid	280
Hydroxybenzoic acid	280

Table 5: Retention times of phenolic compounds present in Ethyl acetate Stem.

Peak	Retention time (min)	Type	Width (min)	Area [nRIU*s]	Height [nRIU]	Area %
1	18.899	BV	1.9375	5.16266e7	3.49883e5	55.2268
2	26.288	VV R	1.4305	4.18544e7	3.93432e5	44.7732

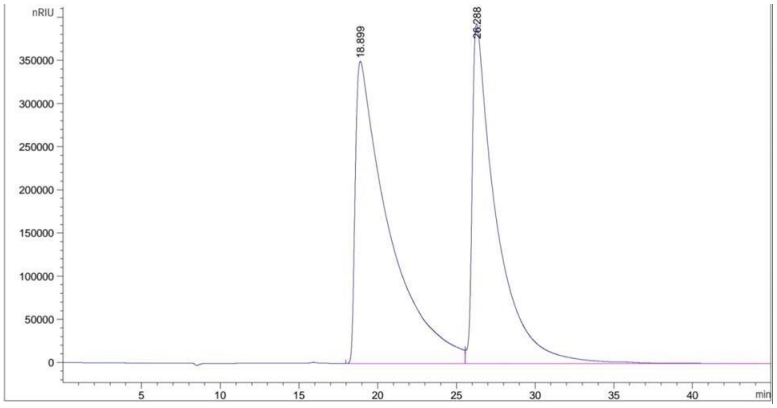


Fig 5: HPLC profiles of phenolic compounds present in Ethyl acetate stem.

Table 6: Retention times of phenolic compounds present in ethyl acetate leaves.

Peak	Retention time (min)	Type	Width (min)	Area [nRIU*s]	Height [nRIU]	Area %
1	19.347	BV	2.0195	5.30699e7	3.44963e5	56.2104
2	26.902	VB	1.4430	4.13430e7	3.87632e5	43.7896

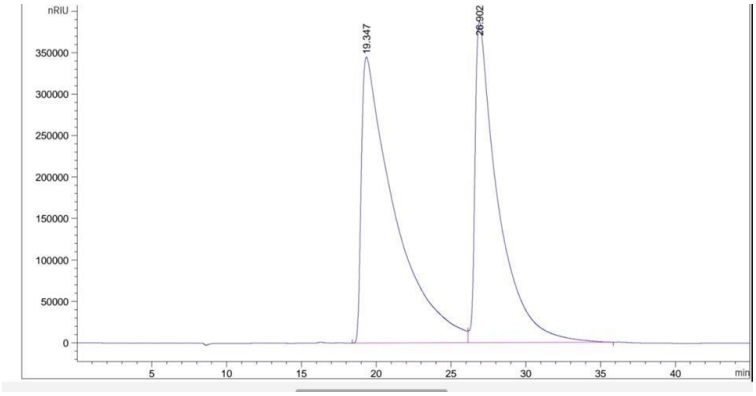


Fig 6: HPLC profiles of phenolic compounds present in ethyl acetate leaves

Table 7: Retention times of phenolic compounds present in ethyl acetate fruit

Peak	Retention time (min)	Type	Width (min)	Area [nRIU*s]	Height [nRIU]	Area %
1	18.908	BV	1.9275	5.14590e7	3.51676e5	55.2835
2	26.283	VV R	1.4270	4.16231e7	3.95142e5	44.7165

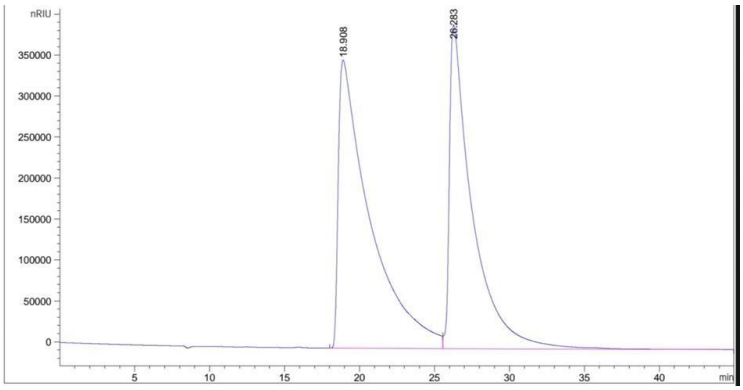


Fig 7: HPLC profiles of phenolic compounds present in Ethyl acetate fruit

Table 8: Retention times of phenolic compounds present in ethanol fruit

Peak	Retention time (min)	Type	Width (min)	Area [nRIU*s]	Height [nRIU]	Area %
1	26.239	BV R	1.2378	3.98304e7	4.99852e5	99.9008
2	42.087	VBAR	0.9748	3.95452e4	521.59802	0.0992

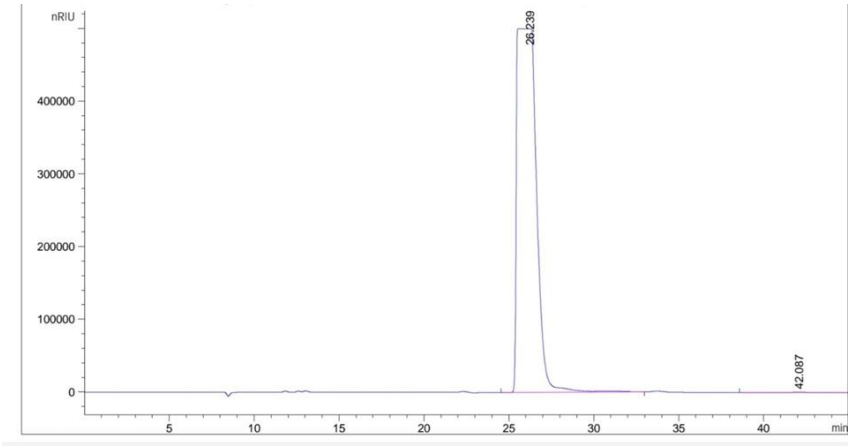


Fig 8: HPLC profiles of phenolic compounds present in Ethanol Fruit

Phenolic compounds were identified in each fraction depicted in Figure (5.11, 5.12, 5.13, 5.14), with peaks displaying varying retention times (RT). The compounds present in Ethyl acetate Stem, Ethyl acetate Leaves, Ethyl acetate Fruit were rutin and vanillic acid. Ethanol fruit had vanillic acid and hydroxybenzoic acid. Vanillic acid was the main phenolic compound identified for the Ethyl acetate extracts whereas it is the most abundant substance in plant extract of *Pyrus pashia.*

CONCLUSION

In this study, phenolic compounds including ascorbic acid, quercetin, gallic acid, resorcinol, catechol, vanillin, and benzoic acid were identified in the fractions of various extracts from Pyrus pashia. It was noted that the ethyl acetate extract exhibited the highest level of pharmacological activity. The present work suggests that *Pyrus pashia* extracts show the capability of a good natural antimicrobial and antifungal agent against infection caused by microbes. Ethyl acetate extract of Pyrus pashia showed the highest activity. *Pyrus pashia* also exhibit high antioxidant activity. A straightforward and accurate HPLC method was devised and validated for the quantification of six phenolic compounds present in plant extracts (gallic acid, catechin, hydroxybenzoic acid, rutin, quercetin, vanillic acid).

REFERENCES

1. Kumar S, Parveen F, Narain P. Medicinal plants in the Indian arid zone.
2. Matin A, Khan MA, Ashraf M, Qureshi RA. Traditional use of herbs, shrubs and trees of Shogran valley, Mansehra, Pakistan. Pak. J. Biol. Sci. 2001;4(9):1101-7.
3. Ghora C, Panigrahi G. The family Rosaceae in India, vol. 2. Bishen Singh Mahendra Pal Singh, Dehra Dun, India. 1995.
4. Kumar M, Sheikh MA, Bussmann RW. Ethnomedicinal and ecological status of plants in Garhwal Himalaya, India. Journal of Ethnobiology and Ethnomedicine. 2011 Dec;7:1-3.
5. Chettri N, Sharma E, Lama SD. Non-timber forest produces utilization, distribution and status in a trekking corridor of Sikkim, india.
6. Murad W, Ahmad A, Gilani SA, Khan MA. Indigenous knowledge and folk use of medicinal plants by the tribal communities of Hazar Nao Forest, Malakand District, North Pakistan. Journal of Medicinal Plants Research. 2011 Apr 4;5(7):1072-86.
7. Chen SB, Liu HP, Tian RT, Yang DJ, Chen SL, Xu HX, Chan AS, Xie PS. High-performance thin-layer chromatographic fingerprints of isoflavonoids for distinguishing between Radix Puerariae Lobate and Radix Puerariae Thomsonii. Journal of Chromatography A. 2006 Jul 14;1121(1):114-9.
8. Cai L, Zhao M, Liu S, Yin T, Zhou H, Dong J, Yang Y, Ding Z. Pashinintide A, the first plant cyclopeptide from Rosaceae, included a sucrose, suggests a new natural receptor for saccharide. Tetrahedron letters. 2014 Nov 5;55(45):6231-5.
9. Muhammad Ibrar Shinwari MI, Mir Ajab Khan MA. Multiple dimensions of ethnobotany and its present status in Pakistan.
10. Gulia KS. Human ecology of Sikkim: a case study of Upper Rangit Basin. Gyan Publishing House; 2005.
11. Manandhar NP. A survey of medicinal plants of Jajarkot district, Nepal. Journal of ethnopharmacology. 1995 Aug 11;48(1):1-6.
12. Kala CP. Medicinal plants of Indian trans-Himalaya. 2003.
13. Murray P, Baron E, Jorgensen J, Landry M, Pealler M. Manual of clinical Microbiology 9th edition" Editorial ASM press. Washington DC, USA. 2007.

14. Khanal LN, Sharma KR, Pokharel YR, Kalauni SK. Assessment of phytochemical, antioxidant and antimicrobial activities of some medicinal plants from Kaski district of Nepal. American Journal of Plant Sciences. 2020 Sep 18;11(09):1383.

15. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958 Apr 26;181(4617):1199-200.

16. Gini TG, Jothi GJ. Column chromatography and HPLC analysis of phenolic compounds in the fractions of Salvinia molesta mitchell. Egyptian Journal of Basic and Applied Sciences. 2018 Sep 1;5(3):197-203.