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Phytochemical screening, antioxidant, and antimicrobial activity of different *Portulaca oleracea* L. extracts growing in Kashmir Valley

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Abstract

This research aimed to evaluate phytochemicals composition, antibacterial, antioxidant, and antifungal activity of different solvent extracts of *P. oleracea* L. (purslane) growing in Kashmir Valley. Different solvent extracts of the aerial parts of *P.* oleracea were prepared including aqueous, methanolic, ethanolic, acetone, and hexane. Qualitative phytochemical analysis was performed by different tests and quantitative analysis for total flavonoids and total phenolics was performed. Antioxidant potential was analyzed through 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, nitro blue tetrazolium (NBT) assay, and FARP assay. Finally, the antimicrobial activity was evaluated through the disc-diffusion method. Results indicated that *P. oleracea* is very rich in phytochemical composition and revealed the presence of phenols, tannins, steroids, flavonoids, alkaloids, saponins, terpenoids, and anthraquinones. Total flavonoids and phenols were found significantly in these extracts while acetone extract showed the highest total flavonoids content (21.75 ± 0.21) and ethanolic extract showed the highest phenolic content (31.97 ± 0.32) mg g^{-1} dry matter (DM). Acetone extract possesses the highest antioxidant activity. The antibacterial activity was analyzed against *S. aereus, E. coli*, and *M. luteus* and antifungal activity against *Aspergillus flavus* and *Fusarium oxysporum*. Each extract showed significant antifungal and antibacterial activity but the highest was revealed by ethanolic extract of *P. oleracea*. Ethanolic extract yielded minimum inhibition concentration (MIC) values of 0.14, 0.05, 0.07, 0.62, and 0.73 mg/ml against *S. aereus, E. coli, M. luteus, Fusarium oxysporum*, and *Aspergillus flavus*, respectively. In conclusion, *P. oleracea* growing in Kashmir Valley showed rich phytochemical composition, strong antioxidant, antibacterial and antifungal activities.

Keywords: Medicinal plants, *Portulaca olerecea* L., Phytochemicals, Antioxidant, Antimicrobial

Introduction

Since time immemorial, plants have been utilized by mankind to serve numerous livelihood requirements (Kanjikar, 2019; Poddar *et al*., 2020). Besides oxygen demand, plants have aided us human beings in different aspects of life including shelter, food, clothing, medicine, etc (Dar *et al.*, 2017; Georgievna *et al.*, 2020; Prasathkumar *et al.*, 2021). Plants are a source of rich chemical compositions like carbohydrates, glycosides, flavonoids, terpenoids, carotenoids, polyphenols, vitamins, proteins, steroids, tannins, etc. (Saxena *et al.*, 2013). The presence of these chemical species makes plants a magnificent part of different traditional medicine systems. Famous medicinal systems like Ayurveda, Traditional Chinese Medicine (TCM), Unani medicine, incorporate several medicinal plants, which prove beneficial in treating several human ailments (Patwardhan *et al.*, 2005). Globally, a high population depends upon plants to fulfill basic needs of life including medicinal needs (Jamshidi-Kia *et al*., 2018). Medicinal plants have been proving beneficial in drug discovery and design (Velmurugan *et al.*, 2020). India is among the vast countries in terms of area and possesses huge floral diversity. India is home to more than 45,000 plant species out of which maximum have been reported of medicinal properties (Mehraj *et al.*, 2018). Kashmir Valley, a division of Jammu and Kashmir Union Territory in India, falls in Westernmost Himalayan ranges. It is known for its extraordinary beauty and high mountain ranges covered with green belt. Due to soothing climatic conditions and nutritious soil quality, Kashmir Valley bears huge floral diversity including several varieties of medicinal plants (Nengroo & Rauf, 2019). *Portulaca oleracea* L. (common purslane) is an agricultural weed growing across the globe and is ranked among top most eight common plants growing on earth (Srivastava *et al.*, 2021). It belongs to the family *Portulacaceae*, which consists of more than 120 succulent shrub and herb species (Zhou *et al.*, 2015). *Portulaca oleracea* L. is locally known as "Nunnar" in Kashmir and is usually found in apple orchids and vegetable crops including carrots, cabbage, cauliflower, spinach, etc. Although purslane is a weed, its stems and leaves are entirely edible bearing a salty taste. In Kashmir, people cook leaves and stems of the weed and eat it with rice. *Portulaca oleracea* L. is of high medicinal value and has been listed among the most used medicinal plants globally by the World Health Organization (Sedaghati *et al.*, 2019). The above ground portions of the plant are a part of folk medicine used in several countries due to its high anti-asthmatic, antipyretic, anti-ascorbic, febrifuge, diuretic, antiseptic, antitussive, vermifuge, and antispasmodic effects (Catap *et al.*, 2018; Alu'datt *et al.*, 2019; Khazdair *et al.*, 2019). Different extracts of the plant have been shown with hypocholesterolemic, bronchodilatory, neuropharmacological, wound healing, analgesic, hypolipidemic, anti-inflammatory, anti-aging, antibacterial, hypoglycemic, antioxidant, and skeletal muscle relaxant (Kaveh *et al.*, 2017; Sallam & Anwar, 2017; Ahmadi *et al.*, 2021). Other studies have

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reported that *Portulaca oleracea* L. shows protective effects against several chronic disorders including heart diseases and cancer (Zhao *et al.*, 2013). The plant is considered an immune booster and suppressor of heart attack due to the high values of omega-3 fatty acids (Simopoulos, 2004). Keeping in the high medicinal value of *P. oleracea*, the present research was designed to assess the phytochemicals, antioxidant, antibacterial, and antifungal activity of different extracts (aqueous, ethanolic, methanolic, acetone, and hexane) of the plant. We performed DPPH, NBT, and FRAP assays to estimate the antioxidant potential of *P. oleracea*. The antibacterial activity of the extracts was evaluated against *S. aureus, E. coli*, and *M. luteus*. Antifungal activity was estimated against *Fusarium oxysporum* and *Aspergillus flavus*.

Materials and Methods

Collection of Plant Material

P. oleracea was collected from apple orchids of Central Kashmir's Chadoora area (33.9451° N, 74.7966° E, elevation of 1,577 m), a tehsil in district Budgam, Jammu, and Kashmir. The plant was authenticated by the Department of Botany, Madhyanchal Professional University, Bhopal, Madhya Pradesh. The aerial plant parts including flowers, stem, and leaves were collected in September and cleaned with tap water.

Sample Preparation

The aerial parts of the plant were shade dried for 3 weeks at a temperature of about 29-30ºC. These parts were cut into small pieces manually and stored in airtight plastic bags to avoid exposure till further use.

Extracts Preparation

The dried aerial parts of *P. oleracea* were incubated at 50ºC for 1 h, in order to remove all the remaining moisture. Then, the plant material was ground thoroughly till a fine powder is obtained. The powder was suspended in the appropriate solvents used in the study (20% w/v): water, methanol (95%), ethanol (95%), acetone (70%), and hexane (70%). For methanolic, ethanolic, acetone, and hexane extractions, suspensions were placed under refluxing fitted with a magnetic stirrer at 20ºC for 48 h. The aqueous extract was prepared by boiling the water and plant powder mixture for 3 h. All the suspensions were allowed to settle down and the liquid phase was passed through filtration with Whatman filter paper no. 1. The filtrate in each case was allowed to dehydrate at 70ºC with a rotatory evaporator. The final crude extracts were prepared by mixing each crude extract with dimethyl sulfoxide (DMSO) to obtain a final concentration of 10 mg/mL. These extracts were then stored at 4ºC until further use.

Phytochemical Screening

Qualitative Analysis

Phenolics

Ferric chloride test was performed to investigate the presence of phenolics in different solvent extracts of *P. oleracea*. 50 mg of extract was dissolved in 5 mL of distilled water followed by the addition of few drops of 5% ferric chloride. This mixture was then examined for the presence of phenolics, which is confirmed by the appearance of Bluish-black color.

Tannins

The ferric chloride test was performed to monitor the presence of tannins in the extracts. Briefly, 50 mg of the plant extract was taken and then dissolved in 10 mL distilled water. This mixture was shaken thoroughly prior to the addition of few drops of ferric chloride (5%). The presence of tannins is confirmed by the formation of a green precipitate.

Steroids

We performed the steroid test to check its presence in the extracts. Briefly, 50 mg of each extracted sample was added to 2 mL of chloroform followed by the addition of 2 mL sulfuric acid. The mixture was allowed to settle down for a few minutes. The presentation of red color by the mixture confirms the presence of steroids.

Alkaloids

The presence of alkaloids in the extracts was monitored by the execution of Wagner's test. In brief, 50 mg from each extract was added with a small quantity of hydrochloric acid followed by filtration. The filtrate was placed in a test tube and added with Wagner's reagent. The Wagner's reagent produces a reddish-brown precipitate in reaction with alkaloids.

Flavonoids

The presence of flavonoids was confirmed by the execution of the alkaline reagent test. About 50 mg of different solvent extracts were added with a dilute ammonia solution (5 mL) and followed by filtration. Then, the filtrate was added with few drops of concentrated sulfuric acid and let to settle down. The yellow color formation reveals the presence of flavonoids in the sample.

Saponins

The presence of saponins in the experimental extracts was confirmed by the Forth test. Briefly, about 50 mg of different *P. oleracea* extracts were dissolved in distilled water to a total volume of 20 mL. This mixture was then thoroughly mixed by continuous shaking for 20 min. The formation of foam to a height of 2 cm from the surface of the mixture confirmed presence of saponins.

Terpenoids

The presence of terpenoids in different plant extracts were confirmed by the Salkowski test. To 50 mg plant material, 2mL chloroform and 3mL concentrated sulfuric acid were carefully added. A reddish-brown coloration signified the presence of terpenoids.

Anthraquinones

30 mg of the extracts and few drops of 10% ammonia solution were added. The appearance of a pink color precipitate indicates the presence of anthraquinones.

Quantitative Analysis

Total Flavonoids

We estimated the total phenol content of different *P. oleracea* extracts via the aluminum chloride spectrophotometric analysis. In brief, 200 µL of each crude extract and/or catechin solution (standard) was added with 1mL distilled water and 70µL of 5% sodium nitrite. Thereafter, this mixture was supplied with 150 µL of aluminum trichloride (10%) and placed over incubation for 10 min. After the incubation period, distilled water and 0.5mL of 1M NaOH was added to this solution to a final volume of 2.5mL. Finally, the absorbance studies were performed at 510 nm against blank. The total flavonoid content in the extracts was expressed in the equivalents of mg (+)-catechin g^{-1} of the dry matter (DM) of the extract.

Total Phenols

We performed the Folin-Ciocalteu method (slight modification) in the determination of total phenols within different extracts of *P. oleracea*. In brief, 100 µL from each extract was mixed with Folin-ciocalteu reagent and deionized water. Then, 2mL of 75% sodium carbonate solution was added to the mixture and left untouched at room temperature for 2.5 h. Finally, the absorbance was recorded at 765nm and total phenols were expressed in terms of mg hydroxytyrosol equivalents g^{-1} of the DM of the extract.

Antioxidant Activity

DPPH Assay

The antioxidant potency of different *P. oleracea* was estimated through 2,2-diphenyl-1picrylhydazyl (DPPH) scavenging efficacy. We prepared the stock solution of DPPH by dissolving 24 mg of DPPH in absolute methanol (100mL). Then, the extract solutions were prepared by dissolving 1 mg/mL of crude extracts in the absolute methanol followed by dilution for the forth analysis. Afterward, we added DPPH (1 mL) to 100 µL of different *P. oleracea* extracts prepared in altering concentrations of 100, 200, 300, 400, and 500 µg/ml. These mixtures were well shaken prior to transfer into a dark room for 15 min at 25 ºC. Finally, the absorbance was taken at 517 nm using methanol as blank. BHT was used as positive control and DPPH activity was determined by the following equation:

$$\%\ Inhibition = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100 \quad (1)$$

NBT Assay

The determination of superoxide anion radical scavenging efficacy of different solvent extracts of *P. oleracea* was analyzed by nitroblue tetrazolium (NBT) reduction. In brief, the reaction mixture comprises of a final volume 3mL Tris-HCL buffer (16mM concentration and pH=8), maintaining 0.3 mM NBT (0.5mL), 0.93 mM NADH (0.5mL) and 1 mL of different solvent extracts with different concentrations viz 100, 200, 300, 400, and 500 µg/ml followed by the addition of 0.5mL of 0.12mM phenazine methosulphate (PMS). The complete reaction mixture was let to settle down followed by 6min incubation at room temperature. Finally, absorbance was measured at 560nm against blank and ascorbic acid was taken as the positive control.

Ferric Reducing/Antioxidant Power (FRAP) Assay

The FRAP method was used to determine the antioxidant activity of different extracts of *P. oleracea*. In brief, 1 ml of each solvent extract with altering concentrations of 100, 200, 300, 400, and 500 µg/ml, were mixed with 2.5 ml of 1% potassium ferricyanide and 2.5 ml of phosphate buffer (pH 6.6). This mixture was subjected to incubation for half an hour at 50ºC followed by the addition of 2.5ml of 10% trichloroacetic acid. Afterward, this mixture was placed for 10 min of centrifugation at 3,000 rpm. The supernatant then evolved was diluted in 2.5 ml of distilled water. This mixture was supplied with 0.5 ml of 0.1% fresh ferric chloride and shaken vigorously. Finally, the absorbance of the samples was taken at 700 nm. Absorbance is directly proportional to the ferric reducing potential of the extracts.

Antimicrobial Activity

Microbial Culture

Five different extracts of *P. oleracea* were investigated for antimicrobial activity against five microbes including *E. coli*, *S. aureus*, *M. luteus*, *Fusarium oxysporum*, and *Aspergillus flavus*. These microbes were collected from the Laboratory of Pharmacy Department, MPU, Bhopal, Madhya Pradesh. Nutrient broth was used to culture bacteria while fungi in PDB (potato dextrose broth) media. All the microbes were cultured for 24 h at 37ºC (bacteria) and 30ºC (fungi) for 24 h.

Antibacterial Assay

Different solvent extracts of the aerial parts of *P. oleracea* were examined for their antibacterial potential via the disc diffusion method. In brief, 10 µl of each solvent extract was prepared in 10% DMSO. These solutions were stored in a chiller till further use. 10^5 CFU/mL (hemocytometer) of the bacterial (*E. coli*, *S. aureus* and *M. luteus*) suspensions were spread gently by using sterile cotton bud over Mueller-Hinton agar. Afterward, discs of 6 mm diameter were sterilized for 20 min at 115ºC followed by the loading of extract concentrations. Streptomycin (30 mg) was used as the positive control. The plates bearing the bacterial cultures were placed with the contact of dried and sterilized discs. The areas were labeled followed by subjecting each disc to incubation for 24 h. Each individual experiment was repeated in triplicates and results were revealed as the diameter (mm) of inhibition zones. The minimum concentration of the extracts that suppressed visual growth of the bacteria was taken as MIC (minimum inhibitory concentration) value.

Antifungal Assay

A similar disc diffusion method was used to examine the antifungal potential of the extracts. Briefly, 10 µl of each solvent extract was prepared in 10% dimethyl sulfoxide (DMSO). These solutions were stored in a chiller till further use. Fungal (*Fusarium oxysporum* and *Aspergillus flavus*) suspensions at a concentration of 10^5 CFU/mL were spread gently by using sterile cotton bud over potato dextrose agar. Then discs of 6mm diameter were sterilized for 25 min at 120ºC followed by the loading of various extract concentrations. Amphotericin-B (25 mg) was used as a positive control. The plates bearing the bacterial cultures were placed with the contact of dried and sterilized discs. The areas were labeled followed by subjecting each disc to incubation for 72 h. Each experiment was repeated in triplicates and results were revealed as % inhibition of diameter (mm). The minimum concentration of the extracts that suppressed any visual growth of the fungi was taken as MIC value.

Statistical Analysis

All the experimental data are expressed as mean±SD of 3 independent replicates. Data analysis was carried out with the help of SPSS 18.0 software. Data comparison was done through one-way ANOVA and significant differences among means were determined by using Tukey's test. The *p*-value of <0.05 was considered statistically significant.

Results and Discussion

Phytochemical Content Within Different Solvent Extracts of P. Oleracea

The plant *P. oleracea* L. (purslane) has been reported to have a rich chemical composition mainly flavonoid, terpenoids, alkaloid, and organic acid (Dabbou *et al.*, 2020). Additionally, fatty acids, terpenoids, vitamins, polysaccharides, proteins, sterols, and vitamins have also been found in purslane (Petropoulos *et al.*, 2016). The phytochemical composition of purslane shows significant variations depending upon a number of factors including harvesting time, environment, culture conditions, and solvent nature used for extraction (Uddin *et al.*, 2014). In this study, we aimed at finding the phytochemical composition of purslane growing in Kashmir Valley. The qualitative phytochemical analysis of different solvent extracts of *P. oleracea* was performed by carrying out different tests. The results **(Table 1)** indicated the presence of phenols, tannins, steroids, alkaloids, flavonoids, saponins, terpenoids, and anthraquinones. The aqueous extract of *P. oleracea* showed positive results for phenols, steroids, alkaloids, tannins, flavonoids, terpenoids, and anthraquinones, and negative for saponins. Methanolic extract showed positive tests for the presence of phenols, steroids, alkaloids, flavonoids, terpenoids, and anthraquinones while negative for tannins and saponins. Ethanolic extract showed positive tests for the presence of phenols, steroids, alkaloids, flavonoids, terpenoids, and anthraquinones while negative for tannins and saponins. Acetone and hexane extracts confirmed the presence of phenols, steroids, alkaloids, terpenoids, tannins, and saponins while turned negative for anthraquinones. Hexane extract turned negative for flavonoids as well. Moreover, we determined the total phenolic and flavonoid content of each extract. The results indicated that purslane is very rich in phenolic as well as flavonoid content **(Table 2)**. Out of the five extracts, methanolic and ethanolic extracts showed higher phenolic content, and acetone and methanolic extracts showed higher flavonoid content, respectively.

Table 1. Phytochemicals present in aqueous, methanolic, ethanolic, acetone, and hexane extracts of *P. oleracea* (aerial parts).

	Different solvent extracts of *P. oleracea* (aerial parts)				
Phytochemicals	**Water**	**Methanolic**	**Ethanolic**	**Acetone**	**Hexane**
Phenols	+	+	+	+	+
Tannins	+	-	-	+	+
Steroids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Flavonoids	+	+	+	+	-
Saponins	-	-	-	+	+
Terpenoids	+	+	+	+	+
Anthraquinones	+	+	+	-	-

The "+" sign denotes "present" and "- " sign denotes "absent"

Table 2. Total phenols and total flavonoids (mg g^{-1} DM) content within different solvent extracts of *P. oleracea*

Phytochemicals	**Water**	**Methanolic**	**Ethanolic**	**Acetone**	**Hexane**
Total Phenols	2.31 ± 0.15	24.36 ± 0.13	21.75 ± 0.21	12.24 ± 0.05	7.4 ± 0.51
Total flavonoids	3.56 ± 0.01	26.55 ± .0.03	25.12 ± 0.11	31.97 ± 0.32	5.5 ± 0.02

Antioxidant Activity of Different Solvent Extracts of P. Oleracea

Several research investigations have revealed that the rich pharmacological and medicinal properties of *P. oleracea* are attributed to its strong antioxidant potential. Phenolic compounds are diversely distributed among plant species and have attracted much attention because of their strong free radical scavenging and antioxidant potency (Hassan *et al.*, 2017). The antioxidant species have revealed tremendous health benefits via their physiological behavior including antioxidant, anticancer, and antimutagenic

(Hassan *et al.*, 2017; Akhtar *et al.*, 2019). Medicinal and dietary plants comprise of high phenolic content and show remarkable inhibition of oxidative stress (Rice-Evans *et al.*, 1996; Tchicaillat-Landou *et al.*, 2018). Total flavonoid and phenol content within plants have been shown to highly determine their antioxidant potential (Ng *et al.*, 2020). *P. oleracea* bears high concentrations of both phenols, as well as flavonoids as revealed above. This indicated that *P. oleracea* could possess very high antioxidant activity. Additionally, *P. oleracea* has been reported to possess higher content of ascorbic acid, omega-3-fatty acids, gallotannins, apigenin, quercetin, kaempferol, and α-tocopherols, which enhances its antioxidant potency (Rahimi *et al.*, 2019). A study performed by Behravan *et al.* evaluated the effects of ethanolic and extract *P. oleracea* on H_2O_2-induced DNA damage in human lymphocytes (Behravan *et al.*, 2011). They performed the comet assay and reported that the aqueous extract remarkably suppressed the DNA damage while no such effect was recorded for ethanolic extract. They concluded that the aqueous extract of *P. oleracea* bears antioxidant components, which could inhibit the induction of oxidative DNA damage against these lymphocytes. Therefore, we investigated the antioxidant activity of different solvent extracts of *P. oleracea* using DPPH scavenging assay, NBT assay, and FRAP assay.

DPPH Scavenging activity

For DPPH scavenging activity, we evaluated different concentrations of aqueous, methanolic, ethanolic, acetone, and hexane extracts of *P. oleracea* viz 100, 200, 300, 400, and 500 µg/ml. equal concentrations of BHT were used as the positive control. The results indicated significant scavenging activity of these extracts **(Table 3)**. The DPPH scavenging activity was observed to increase with the increase in extract concentrations. Acetone extract showed higher scavenging potential with 82.3 ± 1.24% at a concentration of 500 µg/ml. The percentage inhibition of aqueous, methanolic, ethanolic, ethanolic, acetone, and hexane extracts ranged from 13.2 ± 0.22% to 49.6 ± 0.72%, 27.31 ± 0.08% to 78.22 ± 2.18%, 26.44 ± 0.14% to 63.7 ± 1.16%, 34.5 ± 0.16% to 82.3 ± 1.24%, 9.7 ± 0.13% to 45.34 ± 0.19%, respectively. The least activity was shown by hexane extract.

Table 3. DPPH scavenging activity of different solvent extracts of *P. oleracea*.

	% Inhibition					
Extract concentration (µg/ml)	**Water**	**Methanolic**	**Ethanolic**	**Acetone**	**Hexane**	**BHT**
100	13.2 ± 0.22	27.31 ± 0.08	26.44 ± 0.14	34.5 ± 0.16	9.7 ± 0.13	68.35 ± 0.16
200	21.6 ± 0.08	44.85 ± 0.08	34.36 ± 0.08	47.66 ± 0.08	17.4 ± 0.8	74.46 ± 0.08
300	36.43 ± 0.08	57.64 ± 0.14	46.7 ± 0.21	68.47 ± 0.21	32.32 ± 0.8	79.53 ± 0.24
400	41.5 ± 0.04	65.3 ± 0.14	58.4 ± 0.16	74.57 ± 0.16	37.5 ± 0.16	84.98 ± 0.08
500	49.6 ± 0.72	78.22 ± 2.18	63.7 ± 1.16	82.3 ± 1.24	45.34 ± 0.19	89.34 ± 1.8

Nitro-Blue Tetrazolium (NBT) Reducing Activity

Same extract concentrations were evaluated for the NBT assay. Results showed that all the extracts possess strong efficacy to reduce NBT to formazan crystals. The NBT reducing power of each extract was found to be concentration-dependent. Ascorbic acid was used as positive control and showed a percentage inhibition ranging from 63.9 ± 0.02% to 89.367 ± 0.16% at concentrations of 100-500 µg/ml. Acetone and methanolic extracts revealed a strong NBT reducing potential. Aqueous, methanolic, ethanolic, acetone, and hexane extracts revealed a high percentage inhibition at 500 µg/ml viz 51.4 ± 0.17%, 75.25 ± 0.14%, 64.7 ± 0.16%, 80.55 ± 0.24%, 47.34± 0.17%, and 89.367± 0.16%, respectively **(Table 4)**.

Table 4. The NBT reducing potential of different solvent extracts of *P. oleracea*.

	% Inhibition					
Extract concentration (µg/ml)	**Water**	**Methanolic**	**Ethanolic**	**Acetone**	**Hexane**	**Ascorbic acid**
100	12.3 ± 0.14	29.15 ± 0.32	23.66 ± 0.16	32.35 ± 0.16	10.7 ± 0.14	63.9 ± 0.02
200	19.5 ± 0.08	46.52 ± 0.32	31.36 ± 0.14	49.66 ± 0.14	18.54 ± 0.8	74.25 ± 0.14
300	33.43 ± 0.14	56.46 ± 0.16	44.72 ± 0.08	64.45 ± 0.15	33.32 ± 0.14	79.5 ± 0.08
400	42.5 ± 0.02	65.55 ± 0.14	56.9 ± 0.14	71.73 ± 0.18	38.45 ± 0.02	84.63 ± 0.14
500	51.4 ± 0.17	75.25 ± 0.14	64.7 ± 0.16	80.55 ± 0.24	47.34± 0.17	89.367± 0.16

FRAP

The FRAP assay was performed to monitor the antioxidant potency of these extracts. Results showed that each extract showed remarkable potency towards FRAP. The highest FRAP was recorded for ethanolic extract of the plant ranging from 0.5 ± 0.04 to 1.52 ± 0.005 (100-500 µg/ml). Chlorogenic acid was used as a positive control, which showed higher absorbance of 3.58 ± 0.003148 at 500 µg/ml. The results of FRAP analysis of each extract are given in **Table 5**.

Table 5. The FRAP of different solvent extracts of *P. oleracea.*

	Extract concentrations (µg/ml) Absorbance				
Extracts	**100**	**200**	**300**	**400**	**500**
Water	0.55 ± 0.003605551	0.67 ± 0.002081666	0.86 ± 0.004358899	0.98 ± 0.008082904	0.1.08 ± 0.008
Methanolic	0.36 ± 0.042296572	0.57 ± 0.010598742	0.83 ± 0.012858201	0.87 ± 0.009073772	0.98 ± 0.006502525
Ethanolic	0.5 ± 0.04	0.76 ± 0.02	0.94 ± 0.02	1.14 ± 0.009	1.52 ± 0.005
Acetone	0.44 ± 0.004	0.67 ± 0.002	0.86 ± 0.004	0.98 ± 0.004	1.08 ± 0.004
Hexane	0.11 ± 0.003605551	0.27 ± 0.002081666	0.53 ± 0.004358899	0.61 ± 0.008082904	0.72 ± 0.008
Chlorogenic acid	1.088333333 ± 0.042296572	1.742133333 ± 0.010598742	2.57 ± 0.012858201	2.94 ± 0.009073772	3.58 ± 0.003148

Antimicrobial Activity of Different Solvent Extracts of P. Oleracea

Portulaca oleracea L. weed is medicinally as well as pharmacologically important. It has been previously shown with antibacterial, antiviral, and antifungal activity against different microbes (Liu *et al.*, 2021). *P. oleracea* has been reported to induce antifungal effects against dermatophytes (Genera *Trichophyton*) (Oh *et al.*, 2000). Similarly, a study has reported that the methanolic extract of *P. oleracea* inhibited bacterial growth against different gram-negative and gram-positive strains of *Neisseria gonorrhea, Pseudomonas aeruginosa,* and *Escherichia coli.* The inhibition zones (mm) for the gram-negative bacterial strains were found to be 15, 15, and 14 mm, while for gram-positive 15, 14, and 13 mm, respectively. The same study indicated antifungal activity of methanolic extract of *P. oleracea* against *Candid albicans* demonstrating a zone of inhibition of 12 mm (Elkhayat *et al.*, 2008). Key molecular isolations from different parts of the plant have proven beneficial to inhibit virus penetration. Antiviral activity of one such pectic polysaccharide has been reported against herpes virus simplex type 2 via suppression of virus penetration (Dong *et al.*, 2010).

In the current study, we investigated the antifungal and antibacterial activity of different solvent extracts of *P. oleracea* against *M. luteus, S. aureus, E. coli, Fusarium oxysporum,* and *Aspergillus flavus*. The antibacterial activity was examined against one gram-negative strain i.e. *E. coli*, and two gram-negative strains i.e. *S. aureus* and *M. luteus*. The results indicated that all extracts, except hexane, showed potential antibacterial effects against both gram-negative and -positive bacteria **(Table 6)**. Acetone yielded inhibition zones of 13.1 mm, 16.4 mm, and 19.7 with MIC of 0.60 mg/ml, 0.12 mg/ml, and 0.16 mg/ml against gram-negative *E. coli* and gram-positive *S. aureus* and *M. luteus*, respectively. Similarly, Methanolic and aqueous extracts showed inhibition zones of 15.4, 17.4, and 14.3 mm and 12.4, 15.6, and 21.3 mm with MIC values of 0.85, 0.10, and 0.13 mg/ml and 0.70, 0.11, and 0.14 mg/ml, respectively. Ethanolic extract showed the highest antibacterial activity with inhibition zones of 16.7, 21.5, and 23.5 mm against the gram-negative and -positive strains, respectively. Additionally, the MIC values for ethanolic extracts were 0.14, 0.05, and 0.07 mg/ml. Streptomycin used as positive control showed inhibition zones of 28.9, 38.50, 35.40, and MIC values of 0.05, 0.02, and 0.02 mg/ml, respectively. Therefore, ethanolic extract showed comparable antibacterial activity to that of streptomycin.

Table 6. Antibacterial activity of different solvent extracts of *P. oleracea* L. (MIC value expressed in mg/ml).

Extract	*E. coli*		*S. aureus*		*M. luteus*	
	Inhibition zone (mm)	MIC	Inhibition zone (mm)	MIC	Inhibition zone (mm)	MIC
Hexane	NA	-	NA	-	NA	-
Acetone	13.1 ± 0.22	0.60	16.4 ± 0.22	0.12	19.7 ± 0.32	0.16
Methanolic	15.4 ± 0.35	0.85	17.4 ±0.27	0.10	14.3 ± 0.16	0.13
Ethanolic	16.7 ± 0.58	0.14	21.5 ± 0.58	0.05	23.5 ± 0.16	0.07
Water	12.4 ± 0.41	0.70	15.6 ± 0.33	0.11	21.3 ± 0.24	0.14
Streptomycin	28.9 ± 0.26	0.05	38.50 ± 0.25	0.02	35.40 ± 0.34	0.02

Each value is the mean± SD of three biological replicas, NA=No activity, Streptomycin was used as the positive control

The five different solvent extracts of *P. oleracea* were evaluated against *Fusarium oxysporum* and *Aspergillus flavus*. Each of the extracts except water and hexane revealed significant antifungal activity against positive control of Amphotericin B. Acetone extract of *P. oleracea* showed a percentage inhibition of 47.35% and 52.15% with MIC values of 1.80 mg/ml and 1.77 mg/ml against *Fusarium oxysporum* and *Aspergillus flavus*, respectively. Similarly, methanolic extract yielded a percentage inhibition of 49.55% and 55.81% with MIC values of 1.42 mg/ml against *Fusarium oxysporum* and *Aspergillus flavus*, respectively. The positive control showed percentage inhibitions of 8315% and 78% with MIC values of 0.003 mg/ml and 0.0001 mg/ml, respectively. In comparison to the positive control, ethanolic extract showed the highest antifungal activity against *Fusarium oxysporum* and *Aspergillus flavus*. The percentage inhibition was found to be 61.23% and 59.10% with MIC values of 0.62 mg/ml and 0.73 mg/ml, respectively **(Table 7)**.

Table 7. Antifungal activity of different solvent extracts of *P. oleracea* (MIC value expressed in mg/ml).

Extract	*Fusarium oxysporum*		*Aspergillus flavus*	
	% inhibition	MIC	% inhibition	MIC
Hexane	NA	-	NA	-
Acetone	47.35	1.80 ± 0.08	52.15	1.77 ± 0.16
Methanolic	49.55	1.72 ± 0.10	55.81	1.42 ± 0.12
Ethanolic	61.23	0.62 ± 0. 08	59.10	0.73 ± 0.08
Water	NA	-	NA	-
Amphotericin B	83.15	0.003± 0.0001	78.00	0.0025±0.0001

Each value is the mean± SD of three biological replicas, NA= No activity. Amphotericin B was used as a positive control.

Conclusion

The results of the current investigation indicated that *P*. oleracea L. growing in Kashmir has a rich phytochemical composition. The high phytochemical composition is responsible for its diverse pharmacological applications. Qualitative examination of the extracts showed the presence of phenols, tannins, steroids, alkaloids, flavonoids, saponins, terpenoids, and anthraquinones while higher amounts of phenolics and flavonoids were determined in quantitative phytochemical screening. All extracts showed remarkable DPPH scavenging activity, ferric-reducing/antioxidant power, and nitro blue tetrazolium reduction except strong antioxidant activity was shown by acetone extract. These extracts further showed strong antibacterial activity and antifungal activity. The higher antibacterial activity was shown and antifungal activity was shown by the ethanolic extract of *P. oleracea.* Therefore, it may be concluded that *P. oleracea* L. growing in Kashmir possess rich phytochemical composition, which is responsible for its pharmacological behavior and hence can be explored for selective phytochemical analysis and isolations, which could help in future drug discovery and design.

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