Phytochemical Screening and Biological Investigation of Selected Medicinal Plants of District D. I. Khan: A Survey Study

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Abstract

In terms of health and medicine, plants are vital to human existence. In the past, a wide variety of illnesses were treated with a variety of medicinal plants. For a phytochemical analysis, nine distinct medicinal plants were chosen from the isolated parts of District D.I. Khan, Khyber Pakhtunkhwa, Pakistan. These plants are often utilised locally for a variety of medical uses. Leaf samples from locally collected medicinal plants were gathered and utilized in tests after drying in the shade. The antioxidant activity of leaf extracts from collected plants was evaluated using a DPPH scavenging assay. Enzyme activity inhibitory effects were also assessed, as well as antibacterial and antifungal activities against Staphylococcus aureus and Aspergillus Niger, respectively. Amaranthus retroflexes, Winthania somniferous, Marsupium peregrine, and Pergamum harmala, four of the nine species, have demonstrated encouraging outcomes in terms of their antioxidant, antibacterial, antifungal, and anti-diabetic properties. Amaranthus retroflexes had a high potential for treating diabetes, while Winthania somniferous leaf displayed the best DPPH scavenging activity among the plants screened. The best results were obtained with Peganum harmala and Marsupium peregrinum against Aspergillus niger and Staphylococcus aureus, respectively. The research showed that the chosen medicinal herbs had potent antioxidant, antibacterial, and antifungal, as well as anti-diabetic capabilities. Furthermore, it was found that these plants possess antioxidant properties, which may be attributed to the high concentration of phytochemicals present in the samples.

Keywords: Staphylococcus aureus; shade drying; Phytochemicals; Antioxidants; Terpenoids

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INTRODUCTION

The vast majority of medicines used in the past were derived from plants. Since then, higher plants have continued to provide humanity with hitherto unrecognized treatments; today, 50% of all medications used in clinical settings globally are created from natural components (Wadood, 2013). Globally, South Asia is one of several impoverished countries where medicinal plants are an essential component of basic healthcare (Yaseen et al., 2015). Due to their accessibility and social acceptability, traditional drugs are used by about 80% of people in underdeveloped countries (Marwat et al., 2011).

The transition from subsistence use to commercial trade in medicinal plants has led to an increased reliance on wild populations (Malik et al., 2019). This shift poses a significant threat to the survival of all plant species, with medicinal trees being particularly vulnerable due to their slow growth, delayed reproduction, and specific habitat requirements (Khan et al., 2017). The unsustainable harvesting practices, such as cutting down trees without immediate replacement, exacerbate the risk of extinction. Over the years, certain indirect and expert management techniques supported the sustainable use of medicinal plants. However, the advent of urbanization and changes in traditional medical practices have led to the disappearance of these practices, further, jeopardizing the conservation of medicinal plants (van Wyk & Prinsloo, 2018).

Around 10% of people globally have diabetes mellitus (DM), a very common endocrine disorder. Diabetes can be treated topically and orally using anti-diabetic medications such as a-glycosidase inhibitors, biguanides, and sulfonylureas. However, many of these oral anti-diabetic medications have detrimental side effects. Therefore, managing diabetes without experiencing any bad effects is still difficult. These causes have resulted in a rise in the use of complementary therapies, such as therapeutic nutrition therapy, which uses plants as a source of food for therapeutic purposes (Wang et al., 2019). Because modern medicine is so expensive, alternate diabetes prevention and treatment strategies must be created. Traditional medicines extracted from medicinal plants continue to play a vital role in curing DM in developing countries, with approximately 90% of them relying on this traditional practice (Li et al. 2020).

Dera Ismail Khan (D.I.Khan) district is located in the Khyber Pakhtunkhwa province of Pakistan. Lakki Marwat, South Waziristan, Paharpur, Tank, and the northeastern, eastern, and southeasterly directions of the D.I. Khan are nearby. In D.I. Khan the temperature typically ranges between 110- and 120 degrees Fahrenheit. District D.I. Khan is home to several healing plants, including peppermint, herbal, and panir dodi. They are all seasonal plants since some of them are only present at certain times of the year, like the winter or the summer. This study was designed to investigate nine distinct medicinal plants chosen from the isolated parts of District D.I. Khan for phytochemical analysis.

MATERIAL AND METHOD

The complete study was carried out at Gomal University in Dera Ismail Khan, including phytochemical screening, antibacterial activity, antioxidant activity, and anti-diabetic content analysis of medicinal plants.

Study Area

The Dera Ismail Khan district is located in the southern part of Pakistan's Khyber Pakhtunkhwa province (Fig. 1). It comprises Union Council City I and Union Council City II and is centred around the

city of D.I. Khan. The district consists of a total of sixteen Union councils. D.I. Khan shares its borders with Lakki Marwat to the northeast, Tank to the east and southeast, and South Waziristan to the southwest, west, and northwest. In D.I. Khan, temperatures can reach 110-120 °F.

Fig. 1 Map of District Dera Ismail Khan

Collection and identification of plants

Fresh leaf samples of numerous medicinal plants were gathered in District D.I. Khan, including *Withania somnifera, Satureja montana, Maribium peregrinum, Amaranthus retroflexus, Amebia,* and *Peganum harmala*. Each plant specimen was taxonomically identified by Dr. Naimat Ullah, and specimens were added to the Gomal University D.I. Khan under voucher number QA 23457. At Gomal University D.I.Khan, the investigations were conducted in the pharmacy faculty's pharmaceutical laboratory.

Sampling

Each plant's stem and three to five leaves were taken from its natural habitat at four to five distinct sites. The representative sample from each plant was promptly rinsed with tap water to remove any dust, pollutants, or other particles that might have remained. The sample leaves were then completely dried by being left to dry for 4-5 days in the shade at room temperature. An automatic grinder was used to further reduce the size of the dried materials after they had first been ground with a mortar and pestle. Before examination, these powdered samples were packed in plastic bags and kept in the freezer. The first extraction was performed using methanol and ground-up plant materials. After that, was used to evaporate the filtrate's rotary evaporator.

Analysis of the Qualitative Phytochemicals

The following methods were used to identify the presence of several phytochemical classes in the methanolic extracts of plants:

Proteome testing

In addition to the plant extract, 2 ml of a 0.2% Ninhydrin solution was also added. When proteins were present, a violet hue began to appear.

Test for Carbohydrates

Test of Fehling's Solutions

The crude plant extract was combined with equal parts of Fehling's solutions A and B, and then heated. A red-colored precipitate formed, which was a sign of the presence of reducing sugars.

Reagent Test by Benedict

The crude plant extract had been boiled with Benedict's reagent (2 ml) until a reddish-brown colour emerged, indicating the presence of carbohydrates.

Iodine Test

The plant extract was mixed with 2 cc of iodine solution. The appearance of purple or dark blue colours was a sign that carbs were present.

Test for Phenols and Tannins

With the crude plant extract, 2 ml of a 2% $FeCl_3$ solution were combined. Additionally, there was a black or blue-green tinge that indicated the presence of tannins and phenols.

Tests for Flavonoids

Test using Alkaline Reagent

The crude plant extract was mixed with 2% solution of NaOH (2 ml), giving a bright yellow colour. Two drops of diluted acid were added to the mixture, and the colour

faded to colourless, revealing the presence of flavonoids.

Identification saponins

The plant extract was placed in a test tube along with 5 cc of distilled water. When the mixture was vigorously agitated, froth formed, which indicated the presence of saponins.

Tests for Glycosides

The Liebermann Test

About 2 ml of CH_3COOH and 2 ml of chloroform were heated up and added to the plant extract. The appearance of a green colour after cooling the mixture and adding concentrated H_2SO_4 revealed the presence of the aglycone steroidal part of glycosides.

The Salkowski's Test

About 2 ml of a concentrated H_2SO_4 solution was added to the plant extract. Reddish-brown pigmentation indicates the presence of glycosides.

Test of Keller-Kilani

A mixture of 2 ml of glacial acetic acid and 2 drops of a 2% $FeCl_3$ solution was combined with the plant extract and concentrated H_2SO_4 solution. The presence of cardiac steroidal glycosides was suggested by the formation of a brown ring.

Test of Steroids

In addition to 2 ml of concentrated H_2SO_4, the entire plant extract was also mixed with chloroform. The bottom chloroform layer's red colour indicated the presence of steroids. Using 2 ml of concentrated H_2SO_4, acetic acid, and the plant extract combined with 2 ml of chloroform, a second test was conducted. A green hue indicated the presence of steroids.

Terpenoids Test

The plant extract was mixed with 2 cc of chloroform and evaporated in a water bath. After that, 2 millilitres of concentrated H_2SO_4 were added to the residual and heated. A grey hue appeared, which was a sign that terpenoids were present.

Anti-oxidant Activity

DPPH Radical Scavenging Assay

Methanol was used to remove the powdered material from the test plants for 36 to 48 hours. The solvent was evaporated to get crude extracts. 30 minutes of reaction time at room temperature were given to a mixture of 2.5 mL of the methanol extract (100 mg/L) and 1 mL of a DPPH solution in ethanol (0.3 mg/mL). A UV-visible spectrophotometer was used to detect the absorbance at 518 nm. Three times, fresh plant samples were used in the experiment, and each time, the percentage of antioxidant activity was calculated by the methods reported by Shad et al. (2014).

Antimicrobial Activity

Three bacterial strains and two fungus strains were examined in the current investigation for antibacterial and antifungal activities. The fungal strains comprised *A. flavus, A. fumigatus, A. niger,* and *F. solani* species, while the bacterial strains included *Micrococcus luteus, Staphylococcus aureus, Salmonella typhi, Enterobacter aerogens*, and *Salmonella Setubal*. On an agar slant, these strains were incubated at 48°C. To activate them for screening, they were incubated for 24 hours at 37°C on nutritive agar for bacteria or Sabouraud glucose agar (SGA) for fungi, respectively.

Anti-diabetic Activity

Inhibition of Enzymes

The sample was mixed with α-amylase (0.5 mg/ml) at various concentrations (100-500 µg/ml), followed by the addition of 100 µl of 0.2 M phosphate buffer (pH 6.9) and 1% starch solution. After a reaction time of 5 minutes at 37°C, the reaction was stopped by adding 2 ml of the 3,5-dinitrosalicylic acid reagent. The reaction mixture was then heated for 15 minutes at 100°C and subsequently diluted with 10 ml of distilled

water in an ice bath. The amylase activity was determined by measuring the color intensity at 540 nm using a spectrophotometer.

RESULTS

Preliminary phytochemical analysis

The study's focus plants appear to be trustworthy providers of natural resources, according to preliminary phytochemical research. The results demonstrated that the tested/experimented plants exhibited substantial quantities of tannins, flavonoids, steroids, alkaloids, and saponins, while terpenes were found in relatively low amounts (Table 1). The investigation into flavonoids and tannins in the studied plants yielded promising outcomes. The analysis of the six selected medicinal plants yielded positive results for the presence of saponins, tannins, terpenoids, and flavonoids. However, it was observed that these plants had the lowest concentration of steroids in the crude aqueous methanolic extract.

Table 1 Phytochemical analysis of methanolic extract.

Sample Name	Tannins	Flavonoids	Alkaloids	Phenols	Saponins	Steroids
Withania somniferous	++	++	++	++	-	++
Maribuim peregrinum	++	++	++	++	++	++
Amaranthus retroflexus	++	++	++	++	++	++
Peganum harmala	++	++	++	++	++	++

Anti-oxidant activity

DPPH radical scavenging assay

The DPPH radical scavenging experiment was conducted on all the methanolic extracts without any further processing. Bar graphs were used to display the data. According to this study, *W. somnifera* (61.22%) has the highest antioxidant% inhibition activity, followed by *A. retroflexus* (60.90%), *P. harmala* (60.20%), and *M. peregrinum* (55.79%), as shown in Fig. 2.

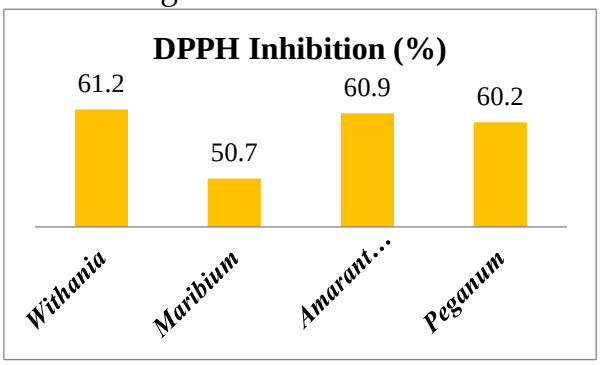

Fig. 2 DPPH scavenging activities of four selected medicinal plants.

Anti-diabetic Activity

In the current study, anti-diabetic effects were carried out on four plants using 3% inhibition of amylase, urease, and lipase. As evidenced by the percent inhibition (46.3%), the analysis showed that *Withania somnifera* had the highest anti-diabetic activity, followed by *Maribium peregrinum* (55.0%), *Amaranthus retroflexus* (54.7%), and *Peganum harmala* (56.3%). Figure 3 displayed the lipase results for *W. somnifera* (48.6), *M. peregrinum* (50.7), *A. retroflexus* (53.2), and *P. harmala* (55.1). In terms of urease percent inhibition, *W. somnifera* ranked at 45.2, followed by *M. peregrinum* (49.5), *A. retroflexus* (55.3), and *P. harmala* (56.2).

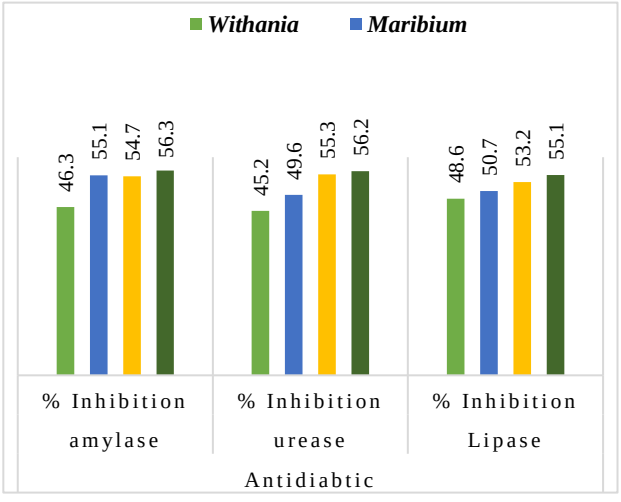

Fig. 3 Analysis of anti-diabetic activity.

Antibacterial Activity

Following the evaluation of the extracts against five different bacterial strains, namely *Micrococcus luteus, Staphylococcus aureus, Salmonella typhi, Enterobacter aerogens,* and *Salmonella setubal*, the inhibitory doses of the effective extracts were determined using the agar dilution method. The results revealed significant inhibition of these bacteria by the plant extract as shown in table 2.

Table 2 Anti-bacterial activity of medicinal plants

Inhibition (mm ± SD)					
Plants Name	M. luteus	S. aureus	S. typhi	E. aerogenes	S. setubal
Withania	11±2.7	12±1.6	12±1.6	12±1.8	11±1.2
Maribium	13±1.6	14±2.7	11±1.2	11±2.7	0.0
Amaranthus	18±1.8	16±2.8	15±1.5	14±2.9	15±2.1
Peganum	12±2.3	11±2.5	12±1.8	11±2.8	11±2.9

Antifungal activity

Following the evaluation of the extracts against five different bacterial strains, namely *A. flavus, A. fumigatus, A. niger, F. solani* and *Mucor* species, the inhibitory doses of the effective extracts were determined using the agar dilution method. The results revealed significant inhibition of these fungi by the plant extract as shown in table 3.

Table 3 Anti-fungal activity of medicinal plants

Inhibition (mm ± SD)					
Plants Name	A. flavus	A. fumigatus	A. niger	F. solani	Mucor specie
Withania	12±1.3	11±2.2	8±2.1	12±1.9	12±2.3
Maribium	11±2.1	8±1.9	Nil	8±0.1	8±2.1
Amaranthus	14±2.4	15±2.4	15±1.9	14±2.8	15±2.2
Peganum	11±1.8	9±0.3	12±3.1	11±2.4	12±2.0
Clotrimazole	35±3.0	31±2.7	34±3.1	25±1.6	24±2.4

DISCUSSION

The current study investigated locally collected medicinal plants that were found in Dera Ismail Khan district of Khyber Pakhtunkhwa for their phytochemical analysis. The study included phytochemical screening, analysis of biochemical components and mineral content, evaluation of antibacterial potential, and assessment of antioxidant activity to investigate for secondary metabolites in *Withania somnifera, Maribuim peregrinum, Amaranthus retroflexus, and Peganum harmala*. The antibacterial potential of the plant extracts was evaluated to determine their ability to inhibit bacterial growth. Additionally, the antioxidant activity of the extracts was measured, highlighting their potential as natural sources of antioxidants. By investigating these parameters, the study aimed to provide valuable information regarding the medicinal properties and potential therapeutic applications of these four plant species. The findings from this research contribute to the understanding of these plants' pharmacological potential and their significance in traditional medicine. We made multiple trips during the research period to speak with the neighborhood herbalists and Hakeems about ethnobotany. In addition to the aforementioned conditions, such as tumors, recurrent fevers, and physical pain, other prevalent ailments in our region, including diabetes, hypertension, and asthma, have been traditionally treated using different parts of various plants. For instance, dried *M. peregrinum* is commonly utilized as a dietary fibre to aid in the management of these conditions. Furthermore, based on the gathered information, *A. retroflexus* has shown effectiveness in treating intestinal bleeding and diarrhoea. *P. harmala* seeds have been traditionally used to alleviate symptoms of

asthma and provide relief from discomfort. The residents were interviewed during the data collection process to gather this valuable information. Further research and exploration are necessary to better understand the specific mechanisms and potential benefits associated with these traditional plant-based treatments for various ailments. The literary analysis further supported its potential for ethnobotany (Ullah et al. 2013). These plants are used by locals on their own for a variety of purposes. Humans primarily appreciate the leaves and stem parts of the plants under study, even if other parts of the plants are employed for varied purposes. The *Withania somnifera* plant, often known as paneer dodi, produces delectable fruit and leaves that also have therapeutic benefits. The blossoms of *Satureja montana* are frequently used to treat a variety of ailments, such as diarrhoea, lung congestion, and irregular menstruation. In District DI Khan, the *Maribuim peregrinum* is abundantly dispersed.

For many years, people have used *amaranthus retroflexus* to treat ailments like liver infections, knee discomfort, stomachaches, diarrhoea, and dysentery. It also functions as a cicatrizing, laxative, and diuretic. The leaves of *A. retroflexes* are consumed as vegetables (Nana et al., 2012). Recent research has demonstrated the potential utility of a powdered infusion decoction derived from *Peganum harmala* seeds in combating tumors, recurrent fevers, and physical pain. These findings highlight the therapeutic properties of this plant extract in addressing these specific health conditions. The infusion decoction made from *Peganum harmala* seeds has demonstrated promise in its capacity to reduce the signs and symptoms of tumours, recurrent fevers, and physical pain (Ullah et al., 2018).

Conclusion and future recommendations

The results of the present investigation demonstrated the medicinal efficacy of all four herbs. Additionally, it was shown that these plants might include anti-diabetic, anti-fungal, antibacterial, and antioxidant compounds. After conducting a chemical study, we have determined that *W. somnifera* exhibits the highest antioxidant activity, while *P. harmala* shows potential for anti-diabetic activity, which could be utilized in diabetes treatment. These findings suggest that both *W. somnifera* and *P. harmala* are reliable sources of antioxidant agents. Furthermore, the ethnobotanical knowledge surrounding these plants supports their traditional medicinal uses. These medicinal plants' antibacterial activity revealed that they may be utilized to create novel antibiotics, and their antifungal activity shows that they can help prevent fungal infections. It is recommended that these plants be included to eliminate health-related issues by proper production, conservation, and chemical research. The results confirmed the need for extensive documentation of the chosen medicinal plants to enable the use of traditional plant knowledge for the treatment of numerous diseases in the future.

AUTHORS CONTRIBUTION

Nasr Ullah Khan and Naimat Ullah conceived the idea, designed the study and drafted the manuscript. Sonia Bibi conducted the experiments and collected and analyzed the data. Sumia Ali and Muhammad Matiullah helped in data collection and analysis.

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