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Research Article

Phytochemical, antimicrobial and elemental status of three anti-diabetic medicinal plants of Balochistan, Pakistan

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Abstract

Phytomedicines are used by tribal communities of Pakistan. This research was aimed to explore variations in total phenolic content (TPC), total flavonoid content (TFC) antioxidant activity (AA), antimicrobial activity and elemental composition of aerial parts of selected medicinal plants *viz.*, *Achillea wilhelmsii*, *Caralluma tuberculata* and *Fagonia bruguieri* used as an anti-diabetic treatment in ethnobotanical knowledge systems of Balochistan. The obtained results showed variation of TPC, TFC and AA in extracts of selected medicinal plants in different solvents *viz.*, Acetone, n-hexane and distilled water. *F. bruguieri* exhibited significant quantities of TPC in hexane extract, and highest concentrations of TFC in aqueous extract. The acetone extract of *C. tuberculata* showed a relatively better AA. Antibacterial activity of plant extracts was evaluated against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method. The results indicated that *A. wilhelmsii* extracts in acetone and hexane were extremely potent against *S. aureus* and somewhat active against *E. coli*. Among the studied elements, maximum concentrations of K in *A. wilhelmsii* and Na in *C. tuberculata* were found. The concentrations Fe, Pb and Mn were found comparatively high. Ni, Zn, Co, Cd, Bi, Cu and Cr respectively were found significantly different and Cd was not detected. These obtained findings revealed that the extracts of selected plants have anti-diabetic potentials due to the presence of bioactive compounds.

Keywords: Antimicrobial; Anti-diabetic; Antioxidant; Elemental; Medicinal plants; Total flavonoid and phenolic

Introduction

Diabetes mellitus, generally referred to as diabetes, is a collection of metabolic disorders characterized by persistently elevated blood glucose levels (hyperglycemia) due to insulin insufficiency, resistance, or both [1]. Insulin is released by beta cells in the pancreas, which convert the glucose from digested food into energy [2]. Hyperglycemia, or an elevated blood glucose level, is a frequent complication of

uncontrolled diabetes that, if left untreated, can cause catastrophic damage to a few the body's organs and most notably the blood vessels and neurons. Over time, it can pose a risk to the heart, kidneys, blood vessels, nerves, and eyes. Diabetes increases the risk of cardiovascular disease and stroke [3]. According to the International Diabetes Federation, around 415 million individuals worldwide were diagnosed with diabetes in 2015, and that number is expected to rise to 642 million by 2040. In Pakistan, one in every eleven persons has diabetes, and 86,364 adults have died due to diabetes [4]. Throughout history, plants have aided globally in discovering therapeutic drugs by providing abundant amounts of particular chemicals necessary for drug development [5]. Nowadays, in developing nations herbal remedies are becoming more popular, because patients prefer natural products, and they are more affordable and widely available. Herbal therapies are especially important as diabetes imposes financial burden on health care systems and national economies [6]. Pakistan is divided into distinct climatic zones and is home to about 6000 plant species, almost 400–600 of which have been investigated for their medicinal properties [7]. However, exploring novel antidiabetic agents derived from natural plants has remained appealing due to phytochemicals such as TPC, TFC and AA. Phytochemicals have demonstrated alternative and safe effects in the treatment of diabetes [8]. Phytochemicals are a diverse category of substances found in plants' secondary metabolites. They have essential roles in plant development and have significant practical applications for nutritive and cosmetic purposes [9]. Plant extracts, individual phytochemicals, or groups of phytochemicals have demonstrated various anti-diabetic effects. These extracts inhibited, expedited, or stimulated a range of processes, lowering the chance of developing diabetes [10]. They have a range of beneficial biological qualities for humans, including antibacterial, antiallergic, anti-inflammatory, anti-diabetic, anticancer, and AA capabilities [11]. Another concern that some diabetes patients suffer is oxidative stress caused by an excessive of reactive oxygen species (ROS) [12]. These reactive oxygen species (ROS) are responsible for the destruction of most macromolecules such as lipids, proteins, and DNA, as well as cell death [13]. Plant components can be utilized to balance the number of AA and free radicals in the body [14]. Investigations into medicinal plants have revealed the presence of key ingredients that can be used for pharmacological or medicinal purposes. The diverse components of these anti-diabetic medicinal plants all have a role in controlling and treating diabetes, either directly or indirectly [15].

The purpose of the presented study was to collect three ethno-medicinal plants used as an anti-diabetic treatment in Balochistan *viz*., *Achillea wilhelmsii* C.Koch (Asteraceae), *Caralluma tuberculata* N.E. Brown (Apocynaceae) and *Fagonia bruguieri* DC (Zygophyllaceae) to explore phytochemical potential, antibacterial activity and elemental composition.

Material and Methods

Plant collection and sample preparation

The aerial parts of the plants (*A. wilhelmsii*, *C. tuberculata* and *F. bruguieri*) were purchased from local market (Pansari shop) selling herbal products/plants and then pulverized using an electric blender. The entire powdered plant was kept at room temperature in airtight plastic containers.

Extraction procedure

A total of 25gm of powdered material was soaked in about 250ml of solvent for three days and then extracted using three different solvents at room temperature: acetone, n-hexane, and distilled water. The plant material was blended 1:2 with each solvent, and then samples were maintained in a shaking water bath at 40°C for 3 hours. After that, flasks were cooled to ambient temperature, then centrifuged for 15 minutes at 4500rpm, and collected the clear

supernatant. The samples were refrigerated for further analyses.

Total phenolic content (TPC)

The TPC was measured using a modified Folin-Ciocalteu reagent technique. A 0.5ml sample was obtained from each extract and diluted with 16.5ml of distilled water in a test tube. 1.0ml of 1:10 Folin reagent and 2.0ml of a 7% sodium carbonate solution were added. After 30 minutes of incubation, its absorbance at 765nm was determined. Gallic acid was used to generate a standard curve.

Total flavonoid content (TFC)

The TFC was determined following Dewanto *et al.* **[16]**. An aliquot of standard solution of catechin was added to a 75 µl $NaNO_2$ solution and stirred for 6 minutes before adding 0.15 ml $AlCl_3$. 0.5 ml NaOH was added after 5 minutes. After adjusting the final volume to 2.5 ml with distilled water and properly mixing, the mixture's absorbance at 510 nm was evaluated compared to a blank of the same mixture.

Antioxidant activity (DPPH Assay)

Two milliliters of the extract at different concentrations were added to 0.5 ml of a 0.2 mM DPPH methanolic solution. After shaking, the mixture was incubated at room temperature in the dark for 30 min, and then the absorbance was measured at 517 nm. Butylated hydroxyanisole (BHA) was used as positive reference while methanol was used as negative reference. DPPH radical-scavenging activity was expressed as the inhibition percentage (I %).

Antimicrobial Screening

The antibacterial activity of the various plant extracts produced via maceration was determined against clinical isolates of *E. coli* and *S. aureus*. The antibacterial test was conducted using a modified Kirby-Bauer disc diffusion technique. To prepare bacterial culture plates, 38g Mueller Hinton Agar (MHA) was dissolved in 1000ml distilled water. At 121°C the mixture was autoclaved for 15 minutes. After allowing the media to cool, it was put into the petri plates. Sub-cultured bacterial strains were placed into petri plates using a sterile swab and left to rest at room temperature. 100µl extracts were placed onto 6mm circular filter paper discs. Together with positive and negative controls, these discs were put on the agar plate. Ciprofloxacin was utilized as a positive control in both tests, while NaCl was employed as a negative control. Plates were incubated at 37°C for 24 hours. The diameter of the zone formed around the discs was used to determine the antibacterial activity.

Elemental analysis

The elemental analyses were done by using atomic absorption spectroscopy (AAS). Nitric acid and Sulfuric acid were used. The samples were boiled and allowed to cool till the completion of digestion until the appearance of white fumes from the flask. Then samples were filtrate by using the filter paper (Whatmann No. 42). The obtained solutions were analyzed by AAS.

Results and Discussion

In present work, the phytochemical attributes of the three therapeutic plants evaluated are shown in (Table 1). The findings indicated that the three plants investigated had medicinally active chemicals. Results indicated that TPC and TFC were present in all plants. The TFC in hexane extracts of all plants was not detected.

TPC were quantified in selected plants while extracts were prepared in different solvents (Table 1). Hexane extracts of *F. bruguieri* contained relatively higher quantities of TPC *viz.*, 0.23±0.02 mg/g whereas *A. wilhelmsii* and *C. tuberculata* showed minor amount 0.03±0.02 mg/g and 0.02±0.01 mg/g respectively. In acetone, *C. tuberculata* showed highest amount of phenolic content 0.14±0.02 mg/g and *F. bruguieri* 0.5±0.02 mg/g and *A. wilhelmsii* 0.06±0.03 mg/g showed least amount comparatively. *F. bruguieri* showed maximum quantity of phenolic compound in distilled water 0.19±0.03 mg/g and moderate amount in *A. wilhelmsii* 0.16±0.01 mg/g while *C. tuberculata* shows least amount 0.09±0.02 mg/g relatively. These results differ significantly from those

previously documented by Masoko & Masiphephethu, [17] The results however contradicted prior reports of Apocynaceae species and showed a greater concentration than that were reported by Wong *et al.* [18]. Additionally, the acquired results are inconsistent with those found by Akhtar and Mirza, [19] in distilled water extracts.

The results of TFC in selected plants of different extracts are shown in (Table 1). Among selected plant species, *F. bruguieri* extract in distilled water showed high content of TFC 9.3±0.5 mg/g whereas *C. tuberculata* extract showed lowest content of TFC 4.5±0.6 mg/g. Similarly, the screening of acetone extracts of *A. wilhelmsii* showed highest TFC 9.2±0.4 mg/g whereas of *F. bruguieri* showed lowest flavonoid content 7.1±0.55 mg/g. Masoko & Masiphephethu show result in TFC whereas in the present study all plants show no results in hexane extracts [17]. The obtained results are greater with those found by Haliloglu *et al.* [20]. The acquired results are nearly conflict with those found by Cacique *et al.* [21].

The AA of the synthetic compound series was determined using the test compound's DPPH free radical scavenging capability. The free radical scavenging capacity of the various extracts against DPPH is summarized in (Table 1). Acetone extracts showed high percentage of DPPH scavenging activity by *C. tuberculata* and *F. bruguieri* 63.10±2.60% and 58.66±2.70% respectively. *A. wilhelmsii* however, showed least DPPH scavenging activity 55.16±2.40%. In hexane extracts, *A. wilhelmsii* indicated highest percentage of DPPH scavenging activity 54.66±2.55% followed by *C. tuberculata* 53.60 ±2.26% respectively. *F. bruguieri* showed least DPPH scavenging activity 50.40±3.31%. *F. bruguieri* and *A. wilhelmsii* showed highest activity 60.13±2.00% and 59.33±2.79% respectively in distilled water. Whereas *C. tuberculata* showed least DPPH scavenging activity 55.70±1.90%. The results however contradicted and showed a greater concentration than that were reported by Akhtar and Mirza, [19]. These values were higher with those mentioned by Abdulsattar and Hossain, [22]. Additionally, the acquired results are consistent with those found by Ahameethunisa and Hopper, [23].

Table 1: Quantitative screening of TPC, TFC (mg/g) and DPPH activity (%age inhibition) of anti-diabetic medicinal plants

S. No.	Medicinal Plant	Acetone	Hexane	Distilled water
1	**TPC (GAE mg/g extract)**			
	A. wilhemsii	0.06±0.03	0.03±0.02	0.16±0.01
	C. tuberculata	0.14±0.02	0.02±0.01	0.09±0.02
	F. bruguieri	0.50±0.02	0.23±0.02	0.19±0.03
2	**TFC (QE mg/g extract)**			
	A. wilhemsii	9.2±0.40	ND	7.2±0.655
	C. tuberculata	7.4±0.40	ND	4.5±0.60
	F. bruguieri	7.1±0.556	ND	9.3±0.50
3	**DPPH (% Inhibition)**			
	A. wilhemsii	55.16±2.40	54.66±2.55	59.33±2.79
	C. tuberculata	63.10±2.60	53.60±2.26	55.70±1.90
	F. bruguieri	58.66±2.70	50.40±3.31	60.13±2.00

Values are expressed as mean of triplicates ± SD, ND-not detected

The antibacterial activity of various extracts of three medicinal plants was determined against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacterial strains in (Table 2). The acetone extract of *A. wilhelmsii* was shown to have substantial antibacterial activity 12±1.153mm against *S. aureus* whereas of hexane extract showed 12±1mm and distilled water extract showed 9±0.8mm

and was less effective than that of acetone extract. *A. wilhelmsii* extract in acetone exhibited maximum antibacterial activity 10±0.9mm against *E. coli* and was less effective in hexane extract 8±0.9mm and distilled water extract 8±0.8mm. Similarly, *C. tuberculata* extract in acetone was found to have a relatively higher antibacterial activity against *S. aureus* 10±1.1mm and least activity was observed in distilled water extract 8±1mm. *C. tuberculata* extract in acetone showed good antibacterial property against *E. coli* 8±1.0mm, whereas relatively low activity in distilled water extract 8±0.85mm was observed and showed no activity against both studied strains of bacteria in hexane extracts. On the other hand, *F. bruguieri* in hexane extract was found to have a maximum antibacterial activity against *S. aureus* and *E. coli* 10±1.1mm and 10±1.0mm respectively. The distilled water extract, however, showed minimum activity against *S. aureus* 10± 0.9mm and *E. coli* 9±0.7mm. *F. bruguier* extract in acetone was inactive against both bacterial strains. Ciprofloxacin was employed as a positive control against both bacteria and had a zone of inhibition of 25mm against *E. coli* and 22mm against *S. aureus*. These findings were nearly identical to those of Alshahrani *et al*. **[24]** and Ahameethunisa and Hopper, **[23]**. These values were in disagreement with those mentioned by Sasikala, Prabakaran and Sabitha, **[25]**. *Tetraena simplex* showed no result in hexane and water extracts against *E. coli* reported by Abdulsattar and Hossain **[22]**.

Table 2: Antibacterial activity of acetone, n-hexane and distilled water extract of anti-diabetic medicinal plants

Medicinal Plant	Bacterial strain Zone of inhibition (mm)					
	S. aureus			*E. coli*		
	Acetone	Hexane	Distilled water	Acetone	Hexane	Distilled water
A. wilhelmsii	12±1.15	12±1	9±0.8	10±0.9	8±0.9	8±0.8
C. tuberculata	10±1.1	ND	8±1	8±1.0	ND	8±0.8
F. bruguieri	ND	10±1.1	10±0.9	ND	10±1.0	9±0.7

Mean of triplicates ± standard deviation; (ND) not detected

The results of various macro and micro elements quantified in selected medicinal plants were listed in (Table 3). The overall results indicated significant concentrations of K in all samples and may suggest these medicinal plants as a good source of K. Present results indicated that K in *A. wilhelmsii* was high *viz.,* 78.8±2.55ppm in HNO_3 extract and lowest in *C. tuberculata* 46.5±2.30ppm in H_2SO_4. Similarly, the concentrations of Na in *C. tuberculata* was 28.13±1.56ppm in HNO_3 and was below the detection limit in *F. Bruguieri.* Highest concentration of Ca was 16.8±1.05ppm and was found in *F. Bruguieri* in HNO_3 and lower levels were detected in *A. wilhelmsii* 7.1±0.6ppm in H_2SO_4 extract. *F. Bruguieri* and *A. wilhelmsii* showed maximum amount of Fe *viz.,* 15.8±0.70ppm and 14.5±0.6ppm in HNO_3 respectively, however *C. tuberculata* 3.8±0.85ppm and *F. Bruguieri* 3.26±0.97ppm showed least concentration in H_2SO_4. Excessive amount of Pb were detected in *F. Bruguieri* 4.52±0.21ppm and least amount were reported in *C. tuberculata* 1.33±0.17ppm in H_2SO_4. The maximum level of Mn content was found in *A. wilhelmsii* which was 2.4 ± 0.2ppm while other plant samples showed moderate concentrations. Ni in *A. wilhelmsii* was 1.76±0.17ppm in H_2SO_4 and in HNO_3 0.32±0.08ppm. *A. wilhelmsii* with 1.16±0.03ppm has maximum concentration of Zn while relatively lower amounts in were found in *F. Bruguieri* 0.29±0.06ppm in HNO_3. *A. wilhelmsii* showed significant concentration in HNO_3 0.27±0.03ppm whereas relatively lower quantity was detected in in H_2SO_4 0.02±0.03ppm. Cd was not detected in *A. wilhelmsii*. Similarly,

Cr remained undetectable, and Co have below the detection limit in all samples. Pb concentrations were more significant than those determined by Anjum *et al*. [26]. The values found for K, Ca, Mn, Zn, Fe, and Cu were inconsistent with those reported by Devi and Sarma, [27]. The concentration of Ca, Mn, Na, Mg, K, Cu, Fe, and Zn were also in disagreement with values obtained by Tkachenko *et al.* [28].

Table 3: Elemental concentration (ppm) of anti-diabetic medicinal plants

Elements	*Achillea wilhemsii*		*Caralluma tuberculata*		*Fagonia bruguieri*	
	HNO_3	H_2SO_4	HNO_3	H_2SO_4	HNO_3	H_2SO_4
Macro elements						
K	78.8±2.55	65.7±2.2	72.46±2.10	46.5±2.30	70.06±1.76	70.0±2.00
Na	17.06±1.40	16.1±1.05	28.13±1.563	12.1±1.1	BDL	BDL
Ca	9.06±0.90	7.1±0.6	12.6±0.81	9.9±0.4	16.8±1.05	8.56±0.65
Mg	1.66±0.66	1.67±0.13	1.67±0.10	1.66±0.10	1.68±0.11	1.64±0.08
Micro elements						
Fe	14.5±0.6	7.7±0.8	9.23±0.83	3.8±0.85	15.8±0.70	3.26±0.97
Pb	2.22±0.20	2.14±0.19	2.70±0.26	1.33±0.17	2.44±0.25	4.52±0.21
Mn	2.4±0.2	1.9±0.12	1.93±0.07	1.4±0.18	1.9±0.09	1.33±0.1
Ni	0.32±0.08	1.76±0.17	0.34±0.09	1.50±0.10	0.53±0.08	1.40±0.09
Zn	1.16± 0.03	0.82±0.07	0.57±0.07	0.32±0.07	0.29±0.06	0.53±0.08
Co	0.27±0.03	0.02±0.03	0.24±0.03	0.14±0.02	0.25±0.02	0.16±0.04
Cd	ND	ND	0.24±0.02	0.1±0.01	0.06±0.01	0.14±0.01
Bi	BDL	ND	ND	ND	BDL	BDL
Cu	BDL	BDL	BDL	BDL	BDL	BDL
Cr	ND	ND	ND	ND	ND	ND

Mean of triplicates ± standard deviation; ND-not detected, BDL-below detection limit

Conclusion

This study provided insight and information for the chemical composition determination of *A. wilhelmsii, C. tuberculata* and *F. bruguieri* utilizing a variety of biochemical assays, with the results validated using a spectrophotometer. The findings showed the existence of medicinally essential components in the plants examined. Numerous research has established that the presence of such phytochemicals confers pharmacological and physiological qualities such as AA, antibacterial, and anti-diabetic capabilities. Three ethnobotanically important medicinal plants of Balochistan were found to have natural AA. They might be utilized to cure various disorders and contribute to preventing these degenerative diseases and the development of new medications. The present investigation detected flavonoid and phenolic components in acetone, hexane, and distilled water extracts and their various fractions. The existence of phytochemical elements in these medicinal plants demonstrates that humans can use them. Additionally, plant extracts can now be used with regular medications to treat various conditions. Each plant has unique active components that help manage blood sugar levels and diabetic problems. In Pakistan, medicinal plants are extracted using a variety of solvents, including ethanolic, methanolic, chloroform, and aqueous. While most studies favored methanolic and ethanolic extracts, the current review demonstrated that nonalcoholic extractions such as n-hexane, acetone, and distilled water also inhibited *E. coli* and *S. aureus* well. One may argue that plants contain various bioactive chemicals, making them a valuable medicinal commodity. However, further research is required to fully characterize its toxicological profile, bioactivity, environmental influence, and agricultural outputs.

Authors' contributions

Conceived and designed the experiments: U Arshad, A Sajjad & S Anjum, Performed the experiments: U Arshad, S Anjum & F Noreen, Analyzed the data: A Sajjad, S Anjum, Z Huma & I Haq, Contributed reagents/ materials/ analysis: A Hussain & I Haq, Wrote the paper: U Arshad & A Sajjad.

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