

Original Research Article

Alpha-glucosidase inhibitory and antioxidant activities of various extracts of aerial parts of *Fagonia indica* Burm. F.

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Abstract

Purpose: To investigate *in vitro* antioxidant and anti-diabetic potentials of *Fagonia indica* Burm.f. Var. *indica* in order to provide scientific evidence for its traditional use.

Methods: Cold maceration method was employed for the preparation of extracts of aerial parts of the plant using chloroform, *n*-hexane, methanol and water as solvents. Total flavonoid and polyphenolic content of various extracts were determined by standard methods. The antioxidant and anti-diabetic activities of the extracts were determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and *in vitro* α -glucosidase (maltase) inhibitory assays, respectively.

Results: Preliminary results indicated the presence of various phytochemicals in the extracts. The chloroform extract exhibited the highest contents of total flavonoids (65.98 ± 1.63 mg QE/g) and polyphenols (26.75 ± 1.09 mg GAE/g). This extract also showed the highest free radical scavenging (64.74 ± 1.43 %) with IC_{50} value of 34.18 ± 5.57 μ g/mL while the methanol extract exerted the highest α -glucosidase (maltase) inhibitory activity (45.22 ± 0.46 %) with half-maximal concentration (IC_{50}) of 220.4 ± 0.41 μ g/mL.

Conclusion: The extracts of the aerial parts of *Fagonia indica* possess significant anti-diabetic and antioxidant effect, thus justifying the traditional use of the plant for treatment of diabetes.

Keywords: α -Glucosidase, Antioxidant, *Fagonia indica*, Flavonoids, Polyphenols

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder which is characterized by an increase in blood glucose level (hyperglycemia) and causes disturbances in fat, protein and carbohydrate metabolism. It causes decline in insulin secretion or its action or both [1]. For the past few years the

increase in the occurrence of diabetes has been observed [2]. The prevalence of diabetic patients has increased from 171 million to 366 million since 2000 and it is expected to rise even more by 2030 [3]. In type 2 DM, starch hydrolysis by the pancreatic α -amylase and the absorption of glucose by the intestinal α -glucosidases

like maltase and sucrase results in postprandial hyperglycemia (PPHG) [4].

Alpha-glucosidase inhibitors suppress the PPHG effect by suppressing the hydrolysis of disaccharides into glucose monomers leading to decreased glucose absorption [5]. However, synthetic α -glucosidase inhibitors like acarbose are often reported with gastrointestinal side effects which include flatulence, abdominal pain and diarrhea [6]. Therefore, it is the need of hour to search for effective and safe alternative α -glucosidase inhibitors showing no side effects. One of the potential approaches to find out a new agent of drug for treating diabetes, especially type 2 DM is the mechanism of α -glucosidase inhibition [7]. Diabetes contributes in causing complications due to free radical reactions [8]. The use of antioxidants in diabetes therapy may prevent diabetic complications [9]. Natural inhibitors of α -glucosidase and α -amylase obtained from the plants can be used effectively in the management of postprandial hyperglycemia having minimum side effects [10]. *F. indica* Burm.f. Var. *indica* (family: Zygophyllaceae) is pruinose glandular or glabrous pale green shrublet having 55 cm height. It is distributed in Pakistan and westwards to North and East tropical Africa [11,12]. It is used as a hypoglycemic agent in a folklore system [13]. Anti-diabetic activity of this plant with a well-defined mechanism has not been evaluated and studied so far. Moreover, no report has shown the antioxidant properties of the plant extracts. This study tends to investigate phytochemicals, the total content of flavonoids, polyphenols, *in vitro* antioxidant and anti-diabetic potential of *F. indica* Var. *indica* to provide the scientific evidence for the folkloric/traditional use of this plant in managing diabetes.

EXPERIMENTAL

Chemicals and reagents

Solvents and reagents of analytical grade were used during the study. 1, 1-diphenyl-2-picrylhydrazyl (DPPH), rat intestinal acetone powder for maltase, Butylated hydroxytoluene (BHT) and FC (Folin-Ciocalteu) reagent were purchased from Sigma-Chemical Co. (USA). Gallic acid and quercetin were purchased from Sigma Life Science (Germany). Methanol, chloroform, n-hexane, hydrochloric acid, copper sulphate, ferric chloride, aluminium nitrate, potassium acetate, sodium hydroxide, acetic acid, sodium phosphate, lead acetate, potassium sodium tartrate and lead acetate were obtained from E. Merck (Germany).

Plant collection and authentication

The aerial parts of the plant were collected from Cholistan desert; Bahawalpur, Pakistan and the plant were authenticated by a plant taxonomist (Prof Dr Zaheer-ud-din Khan, Botany Department, Government College University, Lahore, Pakistan). A voucher specimen (GC-Herb-Bot. 2967) was deposited in Dr Sultan Ahmad Herbarium of the same university.

Sample preparation and extraction

The aerial parts of the plant were air dried and crushed into coarse powder. After drying the powder was extracted sequentially with solvents n-hexane, chloroform, methanol and water of increasing polarity by a cold maceration method with frequent stirring. The filtrates of various extracts were concentrated using a rotary vacuum evaporator (Heidolph, Laborata 4002, Germany) under reduced pressure at 40 °C and were stored at 0 - 4 °C for further use.

Phytochemical evaluation (qualitative chemical examination)

Various extracts of the plant were subjected to a qualitative phytochemical screening to identify phytoconstituents (flavonoids, saponins, terpenoids, phenols, glycosides and alkaloids) responsible for antioxidant and anti-diabetic activities using the standard procedures as described by Trease and Evan, and Sofowara [14,15].

Total flavonoid content (TFC)

TFCs in various extracts were determined following the method of Chang *et al* [16] with minor modification. Stock solutions of quercetin (1 mg/mL) and the extracts (1 mg/mL) were prepared in methanol. Different concentrations (10 - 120 μ g/mL) of the standard quercetin were prepared in methanol. In order to prepare the working solution, 200 μ L was taken in the test tubes for each of the standard and sample, and finally the volume was made up to 1 mL by the addition of methanol. After, 1 M potassium acetate (100 μ L), 10 % w/v of aluminium nitrate solution (100 μ L) and 4.6 mL of distilled water were added to these test tubes. The contents were mixed thoroughly and incubated at room temperature for 45 minutes. The absorbance was measured at λ_{max} of 415 nm using a UV spectrophotometer (UV-1800, Shimadzu, Japan). Blank was prepared similarly as the sample but no sample/standard was added to it. TFC was determined from the standard calibration curve of

quercetin (milligrams of quercetin equivalent/g of plant extract) using linear regression equation.

Total polyphenolic content (TPC)

TPCs of the extracts were investigated following the method described by Slinkard and Singleton [17] with minor modification. Stock solutions of gallic acid (1mg/mL) and the extracts (1 mg/mL) were prepared using methanol as solvent. Concentrations in the range of 10 - 120 µg/mL of the standard gallic acid were prepared to draw the standard calibration curve. 200 µL was taken separately in the test tubes for standard, sample and the volume was made up to 1 mL with methanol. After, 200 µL of FC reagent was added to and the contents, after mixing thoroughly were kept for 4 minutes. Later, the solution of sodium carbonate (15 % w/v, 1 mL) was added and stored at 25°C for 2 h. The absorbance was measured at λ_{\max} of 760 nm wavelength using a spectrophotometer. Blank was prepared similarly, but no standard/sample was added to it. TPC was determined by using calibration curve of standard (milligrams of gallic acid equivalent/g of plant extract) using linear regression equation.

Evaluation of DPPH free radical scavenging activity

The DPPH free radical scavenging activity of the extracts was investigated using DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay as described by Lee and Shibamoto [18] and compared with a standard antioxidant BHT. Samples at concentrations (15 - 75 µg/mL) were dissolved in a methanolic solution of DPPH (3 mL, 0.1 mM). The solutions after shaking vigorously were kept at room temperature for 1 h. Control contained 3 mL of the DPPH solution and was treated like the standard/sample. The absorbance of all extracts, standard and control was measured at λ_{\max} of 517 nm. Methanol was used as blank. Regression equation was applied to calculate IC50 values. Percent free radical scavenging activity (% FRSA) was calculated as in Eq 1 [19].

$$\% \text{FRSA} = \{(Ac - Ae)/Ac\}100 \dots \dots \dots (1)$$

where Ac = the absorbance of control and Ae = the absorbance of extract.

For each extract the assay was repeated thrice and the mean of three values was taken.

Alpha-glucosidase inhibitory assay

The anti-diabetic activity of various extracts was determined by alpha-glucosidase (maltase) inhibitory assay following the standard method of

Dahlqvist [20] with minor modification. Briefly, rat intestinal acetone powder (1 g) was mixed with 10 mL sodium phosphate buffer (pH 7, 0.1 M) and was sonicated for 30 seconds (12 times) with a break of 15 second to prevent heat buildup. Later, centrifugation at 10,000 g at 4 °C for 10 minutes, the resultant supernatant collected was labeled as rat intestinal α -glucosidase (Maltase enzyme). Enzyme inhibition was determined by incubating the solution of an enzyme (20 µL), phosphate buffer (100 µL, 0.1 M) at pH 7.0, maltose solution (37 mM) and the solutions of various extracts of the plant with varying concentrations of 20 - 100 µg/mL at 37 °C for 30 min. Acarbose (a reference standard) at various concentrations (20 - 100 µg/mL) was used as α -glucosidase inhibitor. The mixtures were placed in boiling water for 5 minutes to terminate the reaction. Using the glucose oxidase method of Bergmeyer and Bernt [21], the amount of glucose released was determined. Alpha-glucosidase (maltase) inhibition (H) was calculated using Eq 2 [22].

$$H (\%) = \{(Ac - Ae)/Ac\}100 \dots \dots \dots (2)$$

where Ac = the absorbance of control and Ae = the absorbance of extract.

Statistical analysis

Assays were conducted thrice and the results are presented as mean \pm SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey multiple comparison test (Graph Pad Prism 5.0). Differences were considered to be statistically significant at $p < 0.05$.

RESULTS

Phytochemical profile

Screening of phytochemical constituents of various extracts showed the presence of phytochemicals as shown in Table 1.

Total flavonoid content (TFC)

TFCs of the extracts were determined from the standard quercetin calibration curve using linear regression equation ($Y = 0.001x + 0.0071$) with a correlation coefficient ($R = 0.998574$). The results of TFCs of the extracts are presented in Table 2. It was between 18.891 ± 0.54 mg QE/g to 65.98 ± 1.63 mg QE/g of dried extract. According to Table 2, the chloroform extract showed the highest TFC of 65.98 ± 1.63 mg of QE /g of dried extract. It was followed by n-hexane, methanol and water extracts.

Table 1: Phytochemical screening profile of *F. indica*

Phytoconstituent	HE	CE	ME	AE
Saponins	+	+	+	+
Terpenoids	+	+	+	+
Glycosides	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Phenols	+	+	+	+
Proteins	-	+	+	+
Alkaloids	-	-	+	+
Anthraquinones	-	-	-	-
Carbohydrates	-	+	+	-
Coumarins	+	+	+	+
Fats and oils	+	+	-	-

HE: n-hexane extract; CE: Chloroform extract; ME: Methanol extract; AE: aqueous extract; (-): the absence and (+): the presence of phytoconstituents

Total polyphenol content (TPC)

TPCs of the extracts were determined from the standard calibration curve using linear regression equation ($Y = 0.0037x + 0.0403$) with a correlation coefficient ($R = 0.99736$). The results of TPCs of the extracts are presented in Table 2 and were ranged from 4.96 ± 1.20 to 26.75 ± 1.09 mg of GAE/g of the extract. The chloroform extract showed the highest TPC of 26.75 ± 1.09 mg of GAE/g of the extract which was followed by methanol, n-hexane and water extracts.

Table 2: Total flavonoid and polyphenol contents of plant extracts

Extract	Total flavonoid content (mg of QE/g of dried extract)	Total polyphenol contents (mg of GAE/g of dried extract)
n-Hexane	57.44 ± 1.81	11.67 ± 0.43
Chloroform	65.98 ± 1.63	26.75 ± 1.09
Methanol	24.16 ± 1.27	19.45 ± 0.95
Water	18.89 ± 0.54	4.96 ± 1.20

Values are mean \pm SD (n = 3)

Antioxidant activity

Various extracts of the plant show % DPPH free radical scavenging (Table 3). The results showed that the antioxidant activity increases with increase in concentration of the extract/ sample. Chloroform extract exhibited the highest percentage of scavenging DPPH free radical as compared to other extracts (64.74 ± 1.43 % inhibition at the concentration of $75 \mu\text{g/mL}$).

The IC_{50} (concentration of a plant extract that is required to scavenge 50 % of DPPH free radical) of the extracts are presented in Table 4. Lower the value of IC_{50} , greater will be free radical scavenging activity. The chloroform extract exhibited the lowest value of IC_{50} , i.e., $34.185 \pm 5.57 \mu\text{g/mL}$ compared to the other extracts. The

aqueous extract showed low antioxidant activity, compared to standard (BHT).

Table 3: Percent scavenging of DPPH free radical by various extracts/samples of the plant

Extract	Concentration of extracts ($\mu\text{g/mL}$)	Radical scavenging of DPPH (mean \pm SD)
n-Hexane extract	15	36.21 ± 0.47
	30	42.20 ± 2.87
	45	48.68 ± 2.87
	60	51.79 ± 0.47
	75	57.55 ± 0.43
Chloroform extract	15	42.20 ± 2.87
	30	49.88 ± 1.19
	45	55.63 ± 0.48
	60	61.63 ± 0.71
	75	64.74 ± 1.43
Methanol Extract	15	40.28 ± 0.47
	30	44.60 ± 2.63
	45	51.79 ± 0.47
	60	55.15 ± 2.15
	75	60.91 ± 2.56
Water Extract	15	30.21 ± 0.47
	30	38.36 ± 0.71
	45	44.60 ± 2.63
	60	48.28 ± 2.87
	75	53.23 ± 0.47
Standard BHT	15	48.68 ± 2.87
	30	56.59 ± 0.95
	45	64.74 ± 1.43
	60	78.17 ± 0.71
	75	82.73 ± 0.95

Data are showed as mean \pm SD (n = 3); $p < 0.001$ when compared to control and the standard, acarbose (comparison test)

Table 4: IC_{50} of various extracts of the plant

Standard/extract	IC_{50} of DPPH-radical scavenging ($\mu\text{g/mL}$)
Standard (BHT)	17.82 ± 3.56
n-Hexane extract	43.63 ± 4.73^c
Chloroform extract	34.18 ± 5.57^a
Methanol extract	52.75 ± 4.60^c
Water extract	63.69 ± 4.54^c

Values are expressed as mean \pm SD (n = 3). Values are relative to the standard (BHT); $^a p < 0.05$ and $^{c***} p < 0.001$ in comparison with reference standard (BHT)

Maltase inhibition

The assay showed that various extracts of the plant have α -glucosidase (maltase) inhibition. The methanol extract exhibited the highest maltase inhibition followed by aqueous extract at the concentrations of 20 - 100 $\mu\text{g/mL}$ in a concentration-dependent manner. n-Hexane and chloroform extracts showed minimum maltase inhibitory activity and percentage inhibition increased at a lower level even by increasing the concentration of extracts used.

The maximum concentration of the methanol extract (100 µg/mL) showed the highest maltase inhibition of 45.22 ± 0.46 % and the standard reference (acarbose) exhibited 67.41 ± 0.92 % inhibition at the same concentration (Table 5). It has been suggested that methanol extract is rich in phytoconstituents that have the potential in managing diabetes. The results were evaluated by comparing with the standard (acarbose) as presented in Table 5. A significant decrease in ($p < 0.001$) in the levels of glucose in blood was found among various extracts and standard drug in comparison to diabetic control.

Table 6: IC₅₀ of various extracts

Standard/extract	IC ₅₀ values of maltase inhibition (µg/mL)
Acarbose (standard)	32.51 ± 0.85
n-Hexane	710.69 ± 42.26
Chloroform	579.31 ± 24.48
Methanol	220.40 ± 0.41
Water	236.18 ± 0.85

Results are mean \pm standard deviation ($n = 3$). Maximum concentration taken was 100 µg/mL; *** $p < 0.001$ compared with standard (acarbose)

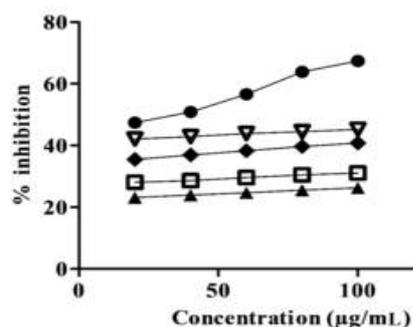


Figure 1: α -Glucosidase (maltase) inhibition by *F. indica* extracts. **Key:** ● acarbose, ▽ methanol extract, ▼ water extract, □ chloroform extract, ▲ n-hexane extract

The IC₅₀ of various extracts and standard (acarbose) are shown in Table 6. The results show that the smaller the IC₅₀ value, the higher

Table 5: α -Glucosidase (maltase) inhibitory activity of *F. indica* extracts

Extract conc. (µg/mL)	Inhibitory activity (%) exhibited by Standard (acarbose)	Inhibitory activity (%) exhibited by n-hexane extract	Inhibitory activity (%) exhibited by chloroform extract	Inhibitory activity (%) exhibited by methanol extract	Inhibitory activity (%) exhibited by aqueous extract
20	47.45 ± 0.77	23.18 ± 0.30	28.12 ± 0.07	42.12 ± 0.23	35.51 ± 0.87
40	50.92 ± 0.46	23.95 ± 0.23	28.65 ± 0.15	42.91 ± 0.23	36.97 ± 0.69
60	56.62 ± 0.92	24.73 ± 1.00	29.66 ± 0.23	43.91 ± 0.30	38.28 ± 0.46
80	63.86 ± 0.84	25.57 ± 0.92	30.50 ± 0.30	44.53 ± 0.61	39.67 ± 0.92
100	67.41 ± 0.92	26.27 ± 0.15	31.12 ± 0.15	45.22 ± 0.46	40.83 ± 0.38

Data are mean \pm SD ($n = 3$); *** $p < 0.001$, compared to diabetic control and positive control (acarbose)

the anti-diabetic activity. Methanol extract was found to have smaller IC₅₀ value in comparison to other extracts. IC₅₀ values of all the extracts showed significant results ($p < 0.001$). The maltase inhibitory activity of various extracts and standard (acarbose) is shown in Figure 1. Inhibition of maltase was observed to be increased with increase in the concentration of the extracts.

DISCUSSION

Keeping the blood glucose level at normal values is a crucial strategy in controlling diabetes with its complications [4]. Therapeutic strategy for the management of diabetes is to decrease PPHG. It is done by the hindrance in glucose absorption by inhibiting carbohydrate metabolizing enzymes (alpha-amylase and alpha-glucosidase) in the gastrointestinal tract. Alpha-glucosidase inhibitors block the action of α -glucosidase enzymes in the small intestine, which limit the conversion of oligo and disaccharides into monosaccharides [23].

The plant under study exhibited α -glucosidase (maltase) inhibition. Methanol extract was found to show the most effective anti-diabetic activity among all the extracts analyzed. Although the extracts (chloroform and n-hexane) have DPPH inhibition (antioxidant activity) at a remarkable level but have less hypoglycemic activity. The study suggests that methanol solvent is efficient to extract hypoglycemic constituents from this plant and this extract exhibiting the maximum activity makes it a target for the isolation and characterization of responsible constituents. DPPH, a free radical has a deep violet colour. It accepts a hydrogen (H) atom or an electron and converts into a stable diamagnetic molecule [24]. The value of absorbance decreases due to the presence of phenolic molecule and the reaction of free radical with antioxidant compound.

This study showed that the chloroform extract, which contains the highest content of flavonoids and polyphenols, exhibited the highest antioxidant activity.

The phytochemical investigation of this plant exhibited positive results for triterpenoids, saponins, flavonoids, phenolics, alkaloids, steroids and tannins. The bioactive compounds obtained from various plants have been reported to have anti-diabetic effects. Triterpenoids, saponins, flavonoids and phenolics have a positive correlation as anti-diabetic agents [25,26].

Previously, anti-diabetic activity of another variety of this plant has been reported in the literature. Ethanol extract (70 %) prepared from the whole plant was evaluated for this study in alloxan-induced hyperglycemic mice. The plant exhibited a significant blood glucose lowering effect (303.86 ± 11.71 mg/dL) when compared with that of the diabetic-untreated group (521.57 ± 17.84 mg/dL) [27]. It can be hypothesized by the results obtained in correlation with the previous reports that there is a significant enzyme inhibition by alcoholic extract.

The anti-diabetic activity shown by the various extracts may be due to saponins, triterpenoids, polyphenols, flavonoids or other phytoconstituents present in the plant. Therefore, further investigations are required to isolate and characterize terpenoids, saponins or other phytoconstituents responsible for α -glucosidase inhibition.

CONCLUSION

F. indica Var. *indica* possesses anti-diabetic activity. Thus, the plant is a potential source of effective anti-diabetic therapeutic agent. Furthermore, the results lend some support for the traditional use of this plant in the management of diabetes mellitus.

DECLARATIONS

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Conflicts of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this research work was done by the authors named in this article and the liabilities pertaining to claims relating to the content of this article will be borne by the authors. The study was conceived and designed by Prof Dr Abida Latif. The plant was collected by Atiq-ur-Rehman and dried and pulverized by Dr. Imran Waheed. Experimental work was conducted by Atiq-ur-Rehman and Dr Atta-ur-Rehman. Data handling and statistics application was done by Dr Muhammad Naeem Qaisar. The manuscript was written by Atiq-ur-Rehman and Dr Abida Latif. Dr Nasir Abbas has finally reviewed the manuscript. All authors read and approved the manuscript for publication.

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