In-vitro antioxidant, antidiabetic activities and phytotoxic profile of *Alkanna tinctoria* (Boraginaceae)

Abstract

Plants are the most important source ~~of source~~ of biological compounds that are used to achieve biological activity. The originating of crude extracts of plants is broadly used as therapeutic drugs to treat various infectious damages.~~disease infections~~. The p~~P~~resent study was performed to investigate the potential of *Alkanna tinctoria* Tausch, roots extract from district Bannu, Pakistan in contradiction of various human pathogenic infections, resistant and antioxidant activities like DPPH (2, 2- diphenyl-1-picrylhydrazy) free radicals scavenging, hydrogen peroxide scavenging, ABTS [2, 2-azinobis (3-ethylbenzthiazoline-6-sulphonic acid)] scavenging activity and p~~P~~hytotoxicity effect. The current study was focused on the phytochemical investigation and pharmacological activities of methanolic extract of *Alkanna tinctoria*. The plant extracts were examined for antioxidant scavenging assay by using DPPH ~~(2, 2- diphenyl-1-picrylhydrazy)~~ & ABTS ~~[2, 2-azinobis (3- ethylbenzthiazoline-6-sulphonic acid)]~~ free radicals, Hydrogen peroxide, alpha amylase and Phytotoxicity. Ascorbic acid was taken as standard. Methanolic crude extract showed high phenolic contents and significant antioxidant activity (DPPH, H_2O_2 and ABTS). All the concentration of *Alkanna tinctoria* also showed p~~P~~hytotoxicity effect and was found to have significant potential.

Keywords: *Alkanna tinctoria*, phytochemical investigation, pharmacological activities, DPPH, H_2O_2, ABTS and Phytotoxicity.

Introduction

Medicinal p~~P~~lants are the most important source of biological compounds. According to World Health Organization (WHO) in 91 countries there are nearly 2000 medicinal plants. Medicinal plants are still being used by rural communities with increasing popularity for treating or preventing various infections. In Pakistan limited data are~~is~~ available on the therapeutic uses of medicinal plants. *A~~a~~lkanna tinctoria* Tausch, "alkanet", is under exploration as having numerous beneficial uses (Ahvazi *et al~~t~~.*, 2012), though has not been recognized for its anti-drug resistant anti-bacterial activity and phytochemical~~s~~ ingredients. Current occurrence of antibiotic resistance activate

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research to discover therapeutic plants for their antimicrobial safety against MDR (multi-drug resistant pathogens) because there is need to discover new antibiotic therapies to combat resistance to older drugs. Hospital picked up infections caused by MDR bacteria is main trial for clinician. Now e days most of peoples believe that medicinal plants are the backbone of traditional medicines and play an important role in food and used as first health care treatment of various infections (Khan *et al*., 2015).

The present study was carried out the phytochemical investigation, pharmacological activities, DPPH, H_2O_2, ABTS and Phytotoxicity of *Alkanna tinctoria* roots extract.

Materials and Methods

1. DPPH Scavenging activity

The DPPH activity was performed following the (Bibi *et al*., 2011) methods. For preparation of DPPH solution 5g of metabolic extract was dissolved in 100 ml methanol in 300 ml volumetric beaker. Various concentrations of solvent extract (25, 50, 50 and 100 mg/ml) were formed in DPPH solution. Optical density of spectrometer was fixed at 517 nm. The extract concentrations were made in test tube and were incubated for 10-15 min, taking the Ascorbic acid ~~was taken~~ as a standard.

$$\textbf{Scavenging effect \%} = \frac{[\text{Control absorbance} - \text{sample absorbance}] \times 100}{\text{Control absorbance}}$$

2. Hydrogen peroxide scavenging activity

To determine the hydrogen peroxide value of plant extract having different concentration (0.37, 0.75 and 1.5mg/ml) was performed by using the (Wasiullah *et al*., 2019) method. Two~~2~~ ml H_2O_2 solution was formed in 50 ml phosphate buffer having pH 7.4. The extract sample (0.1) was poured into a test tube and was diluted up to 0.4 with 50ml phosphate buffer. The hydrogen peroxide solution was added to each test tube of plant extract sample. The absorbance of spectrophotometer was set at 240 nm and was incubated for 10 min.

$$\textbf{H}_2\textbf{O}_2\textbf{ Scavenging activity \%} = \frac{[1 - \text{absorbance of sample}] \times 100}{\text{Control absorbance}}$$

3. ABTS free radical scavenging activity

The ABTS is the abbreviation of 2, 2, axobis, 3-ethyl benxothioxoline-6-sulphonic acid. The ABTS scavenging activity is carried out by changings minute. The ABTS solution was added to potassium per sulphate 2.45 mM solution and was placed in the dark over whole night gain radical cation. The solution was further diluted to gain initial absorbance of 0.811 at 745 nm. During this time the temperature should 30°C. There were m~~M~~ixed 0.2 ml sample with 0.8 ml ABTS standard in a micro cuvette. At the time of

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Did you use the plant complete or only roots?.

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[(control absorbance - sample absorbance) / control absorbance] x100

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[(1-absorbance of sample) / control absorbance] x100

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adding the absorbance was starting decreasing. The absorbance was checked from 1-6 min and was calculated. The percentage effect was calculated by the following formula.

$$\textbf{ABTS Scavenging effect \%} = \frac{[\text{Control absorbance - sample absorbance}] \times 100}{\text{Control absorbance}}$$

4. Alpha- amylase activity

Alpha amylase activity is used for the determination of in-vitro antidiabetic activity of samples. In-vitro-amylase inhibitory activity of plant extract was determined by using the (Malik, 1981) procedure. 0.1 mg~~m~~ (0.1% w/v) potato starch was dissolved in 100 ml sodium acetate buffer. The enzyme solution was formed by dissolving 300 µl of amylase in 700 µl distilled water. These solutions were used as a calorimetric reagent. Four different concentrations (25, 50, 75 and 100 ml) of plant samples were prepared from stock solution of plant extract. The potato starch and amylase solution was added to each concentration and then was incubated for 10 min at 25°C. After incubation in potassium sodium ~~potassium~~ tartrate~~s~~ reagent, and 3, 5 di-nitro salicylic acid was added to each concentration. Distilled water was used as a standard (control). The experiment was repeated two times and the percentage inhibition was calculated by using the following formula:~~-~~

$$\textbf{~~\%~~ Inhibition \%} = \frac{[\text{Control absorbance - sample absorbance}] \times 100}{\text{Control absorbance}}$$

5. Phytotoxicity assay

For determination of Phytotoxicity assay (Mclaughlin and Roger~~s~~, 1998) was used. The alle~~i~~lopathy/ phyto lethal was run P~~p~~etri plates mode. Autoclaved P~~p~~etri plates were set with filter paper. The assay was run in duplicate for each concentration. Different concentrations (100, 250, 500 and 1000 µl/mg) of plant extract was prepared and sprayed in separate P~~p~~etri plates. The plates were placed in oven for drying. After drying the plates were sprayed with distilled water. Before placing the seeds in P~~p~~etri dishes~~plated~~ they were soaked in water for one hour. Seeds were placed at equal position. Petri plates were incubated at 25°C. The f~~F~~irst reading was taken after 3 days by graduated ruler at both shoot and root length with respect to control and the mean was taken of each concentration. The 2nd reading was taken after 7 days and designed the result.

Results and Discussion

Many researchers in the past have proved that plants are the potential source of therapeutic medicines. Medicinal plants Antioxidant activity of methanolic extract of *Alkanna tinctoria* was performed against various free radicals. DPPH activity was performed following the (Bibi *et al*., 2011).

1. DPPH scavenging free radicals effect

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The result obtained from different concentrations of methanolic extract of *Alkanna tinctoria* showed significant decreasing in DPPH scavenging free radicals effect. *Alkanna tinctoria* DPPH scavenging effect is shown in Table 1. ~~Ascorbic acid was used as standard. In present study it was concluded that plant extract of *Alkana tinctoria* was efficient in decreasing the DPPH free radicals. It shows that *Alkana Tinctoria* is active in scavenging DPPH free radicals . Alkana Tinctoria DPPH scavenging effect is shown in Table 1.~~

2. Hydrogen peroxide (H_2O_2) activity

Hydrogen peroxide scavenging activity was measured by the method (Wettasinghe and Shahidi., 2000). Hydrogen peroxide activity was carried out with different dilution of *Alkanna tinctoria*. All the concentrations of extract of plant of *A. ~~lkana~~ tinctoria* showed significant scavenging effect ~~as~~ (Table 1).

3. ABTS free radicals scavenging activity

Plant extracts exhibit antioxidant properties and is therefore rich sources of natural antioxidants. This result accord well with (Popovic *et al*., (2006) and; Ciz *et al*., (2010). The ABTS assay is used to show the scavenging decreasing effect of free radicals. In our result it was investigated that the plant *Alkanna tinctoria* shows significant decreasing and efficiency in scavenging ABTS free radicals. In ABTS assay ascorbic acid was taken as standard in a compares of effect of plant extract. *Alkana tinctoria* showed remarkable scavenging ABTS effect with enhancing in the range from 0.45 mg/ml, 0.75 mg/ml, and 1.7 mg/ml to 4 mg/ml respectively (Table 1).

4. Alpha amylase activity

Alpha amylase activity was performed for the determination of in-vitro anti-diabetic activity of *Alkanna tinctoria* plant extract. In-vitro-amylase inhibitory activity of plant extract was determined by using the (Malik, 1981) procedure Anti-diabetic activity was carried out with different dilution of *A. ~~lkana~~ tinctoria* plant extract. Significant inhibition and antidiabetic activity was found in plant extract as a compared to control ascorbic acid (Table 1). Ascorbic acid was used as a reference ~~as shown in Table 1~~.

5. Phytotoxicity assay

The Phytotoxicity of plant *Alkanna tinctoria* roots and shoots extract is shown in Figure 2. Phytoxicity activity was carried out on the root and shoots of plant by using different concentrations (100, 250, 500 and 1000 µl/mg) of *A. ~~lkana~~ tinctoria* extract was dissolved in different methanolic concentrations. Result was obtained at 3 and 7 days of the experiment and the weight of dry roots and

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shoots were measured as and were showed that there was no growth inhibition in the roots and shoots of the plant ~~as shown in~~ (Table 2).

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You must explain more the results and It would be interesting (if it is possible) to do more discussion

Conclusions

The plant under study was selected to perform ~~plant was for~~ Antioxidant DPPH, Antioxidant ABTS, Hydrogen peroxide (H_2O_2) Alpha- amylase activity and Phytotoxicity activity. The s~~S~~ummary of the data achieved showed that methanolic crude extract of plant showed good antioxidant activity: ~~Antioxidant~~ DPPH, ~~Antioxidant~~ ABTS, Hydrogen peroxide (H_2O_2) activity. ~~.~~ Significant inhibition and antidiabetic activity was found in plant extract. Phytotoxicity of plant showed no inhibition in growth. A significant alpha amylase inhibition was measured against to ascorbic acid.

Table 1. Scavenging effect and Alpha-amylase inhibition of *Alkanna tinctoria* roots extract.

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Concentration	DPPH free radicals	H_2O_2 scavenging	ABTS scavenging ability	Alpha-amylase inhibition %
0.37mg/ml	44.55	66.55	66.55	45.36
0.75mg/ml	63.57	70.65	70.65	63.45
1.50 mg/ml	67.07	75.35	75.35	79.89

Table 2. Phytotoxicity assay of *Alkanna tinctoria* roots extract.

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Concentration	Roots growth inhibition		Shoot growth inhibition	
	3 days	7 days	3 days	7 days
100µl/mg	4.2	6.2	3.9	4.8
250µl/mg	3.3	5.0	2.3	4.0
500µl/mg	3.0	4.5	1.6	2.8
1000µl/mg	2.7	3.0	0.9	1.5

NOTE:

The study highlights the efficacy of "traditional medicines" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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References **Take care to cite references. You must get the uniformity in the cites and following the Journal guidelines for authors**

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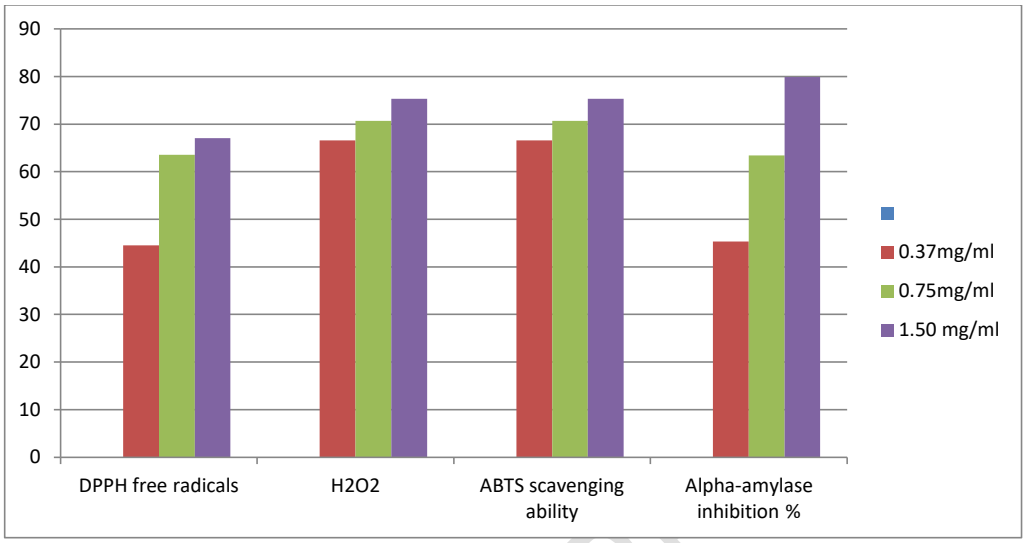

Figure 1. Scavenging effectac and Alpha-amylase inhibition assay of *Alkanna tinctoria* roots extract.

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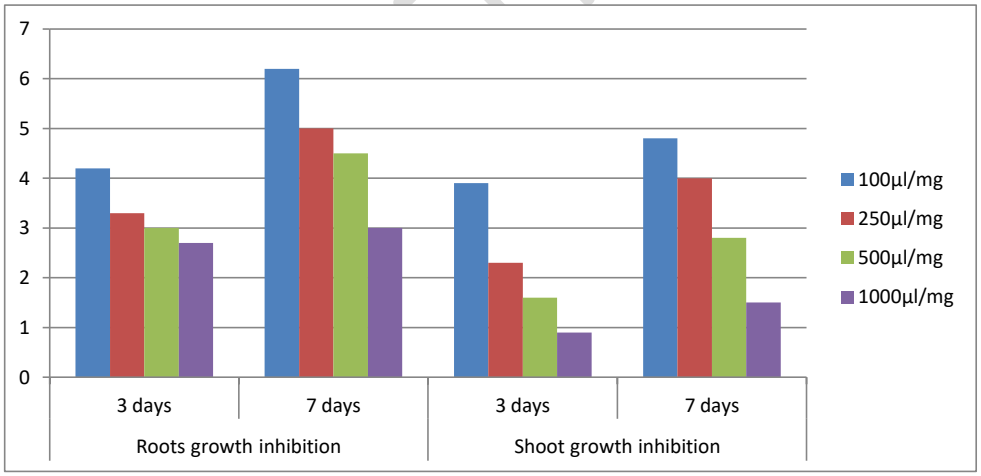

Figure 2. Phytotoxicity assay of *Alkanna tinctoria* roots and shoot extracts.