

In vitro* antioxidant, antidiabetic activities and phytotoxic profile of *Alkana tinctori

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 disease infections. Present study was performed to investigate the potential of *Alkana tinctoria* roots extract from district Bannu, Pakistan in contradiction of various human pathogenic infections, resistant and antioxidant activities like DPPH free radicals scavenging, hydrogen peroxide scavenging, ABTS scavenging activity and Phytotoxicity effect. The current study was focused on the phytochemical investigation and pharmacological activities of methanolic extract of *Alkana tinctoria*. The plant extracts were examined for antioxidant scavenging assay by using DPPH (2, 2- diphenyl-1-picrylhydrazyl) & ABTS [2, 2-azino-bis (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, H₂O₂ and ABTS). All the concentration of *Alkana tinctoria* also showed Phytotoxicity effect and was found to have significant potential.

Keywords: *Alkana tinctoria*, phytochemical investigation, pharmacological activities, DPPH, H₂O₂, ABTS and Phytotoxicity

Introduction

Medicinal Plants 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 is available on the therapeutic uses of medicinal plants. Among these medicinal plants *alkana tinctoria* is under exploration as having numerous beneficial uses (*Ahvazi et al., 2012*), though has not been recognized for its anti-drug resistant bacterial activity and phytochemicals ingredients. Current occurrence of antibiotic resistance activate research to discover therapeutic plants for their antimicrobial safety against MDR 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, H₂O₂, ABTS and Phytotoxicity of *Alkana tinctoria* roots extract.

Material and Methods

1. DPPH Scavenging activity

DPPH activity was performed following the (*Bibi et al., 2011*) methods. For preparation of DPPH solution 5g of metabolic extract was dissolved in 100ml methanol in 300ml 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 517nm. The extract concentrations were made in test tube and were incubated for 10-15min. Ascorbic acid was taken as a standard.

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

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. 2 ml H₂O₂ solution was formed in 50ml 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 240nm and was incubated for 10 min.

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

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 2.45mM 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 745nm. During this time the temperature should 30°C. Mixed 0.2ml sample with 0.8ml ABTS standard in a micro cuvette. At the time of 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.

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

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. 0.1gm (0.1% w/v) potato starch was dissolved in 100ml sodium acetate buffer. The enzyme solution was formed by dissolving 300µl amylase in 700 µl distilled water. These solutions were used as a calorimetric reagent. Four different concentrations (25, 50, 75 and 100ml) 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 at 25°C. After incubation sodium potassium tatrates and 3, 5 di-nitro salicylic acid was added to each concentration. Distilled water was used as a standard. The experiment was repeated two times and the percentage inhibition was calculated by using the following formula.

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

5. Phytotoxicity assay

For determination of Phytotoxicity assay (Mclaughlin and Roger., 1998) was used. The allilopathy/phyto lethal was run petri plates mode. Autoclaved petri plates were set with filter paper. The assay was run in duplicate for each concentration. Different concentrations (100, 250, 500 and 1000µl/mg) of plant extract was prepared and sprayed in separate petri plates. The plates were placed in oven for drying. After drying the plates were sprayed with distilled water. Before placing the seeds in petri plated they were soaked in water for one hour. Seeds were placed at equal position. Petri plates were incubated at 25°C. First 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 *Alkana tinctoria* was performed against various free radicals. DPPH activity was performed following the (Bibi et al., 2011).

DPPH scavenging free radicals effect

The result obtained from different concentrations of methanolic extract of *Alkana tinctoria* showed significant decreasing in DPPH scavenging free radicals effect. 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.

Hydrogen peroxide (H₂O₂) activity

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

ABTS free radicals scavenging activity

Plant extracts exhibit antioxidant properties and is therefore rich sources of natural antioxidants (Popovic et al., 2006; Ciz et al., 2010). ABTS assay is used to show the scavenging decreasing effect of free radicals. In our result it was investigated that the plant *Alkana 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.

Alpha amylase activity

Alpha amylase activity was performed for the determination of in-vitro anti-diabetic activity of *Alkana 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 *Alkana tinctoria* plant extract. Significant inhibition and antidiabetic activity was found in plant extract as a compered to control ascorbic acid. Ascorbic acid was used as a reference as shown in Table 1.

Phytotoxicity assay

The Phytotoxicity of plant *Alkana Tinctoria* roots and shoots extract is shown in fig. Phytotoxicity activity was carried out on the root and shoots of plant by using different concentrations (100, 250, 500 and 1000µl/mg) of *Alkana 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 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.

Conclusions

The plant was selected plant was for Antioxidant DPPH, Antioxidant ABTS, Hydrogen peroxide (H₂O₂) Alpha- amylase activity and Phytotoxicity activity. The Summary of the data achieved showed that methanolic crude extract of plant showed good antioxidant activity Antioxidant DPPH, Antioxidant ABTS, Hydrogen peroxide (H₂O₂) 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 *Alkana tinctoria* roots extract

Concentration	DPPH free radicals	H ₂ O 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 *Alkana tinctoria* roots extract

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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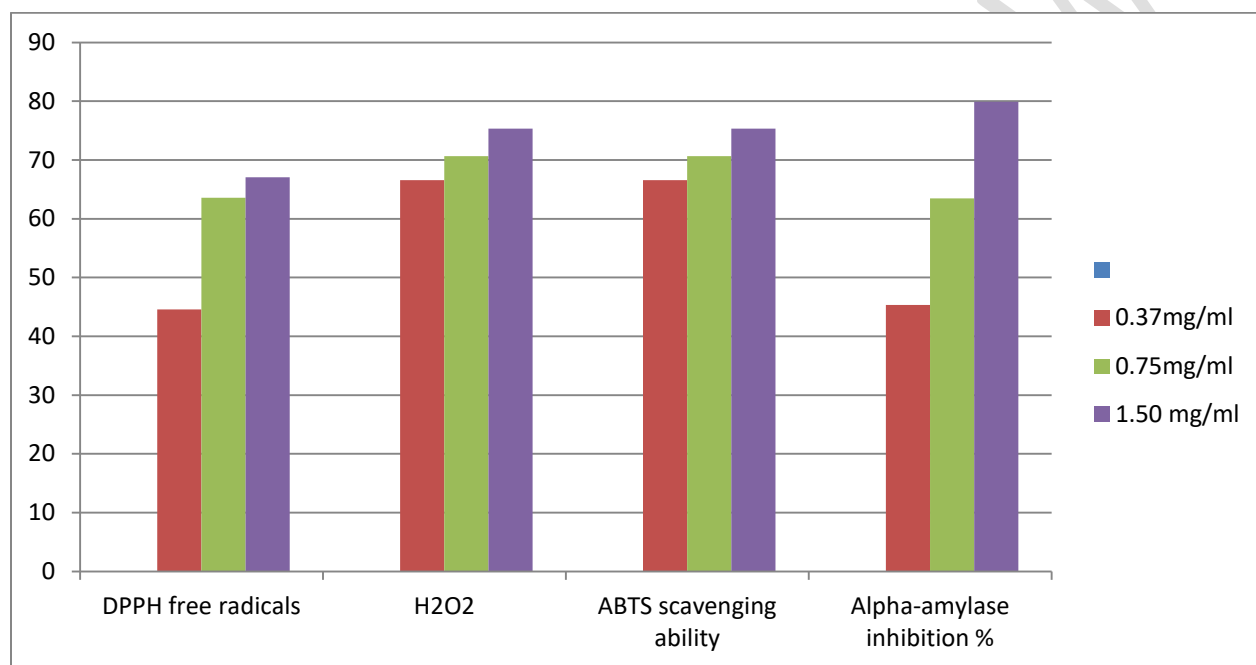


Figure 1. Scavenging effect and Alpha-amylase inhibition assay of *Alkana tinctoria* roots extract

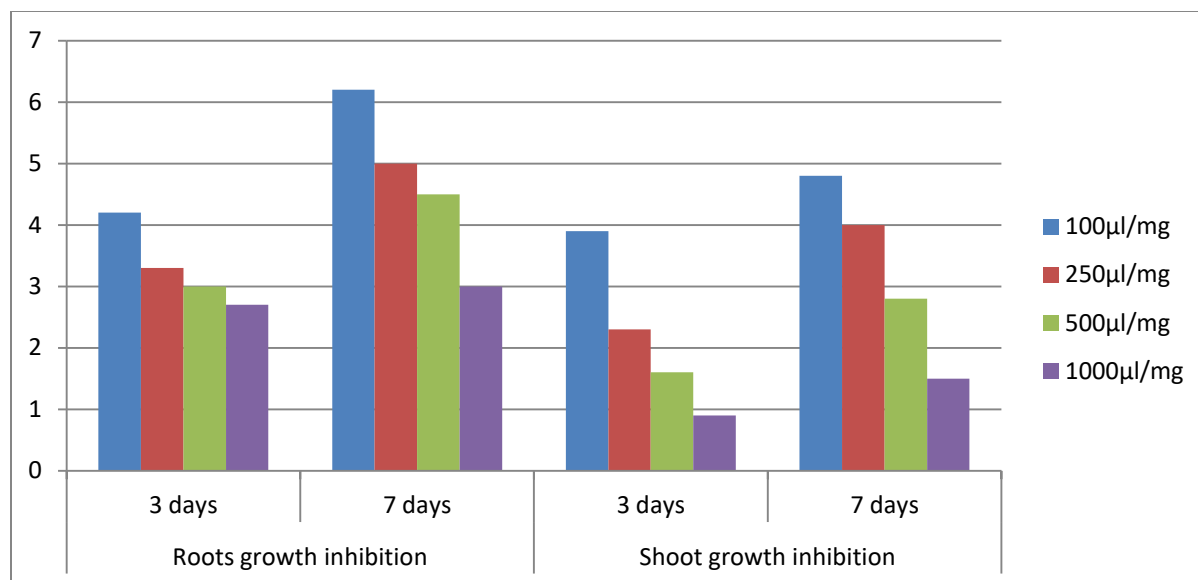


Figure 2. Phytotoxicity assay of *Alkana tinctoria* roots extract

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