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

Abstract

Plants are the most important 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. The present study was arranged to investigate the potential of *Alkanna tinctoria* Tausch, roots extract, collected 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 phytotoxicity 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 & ABTS 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 phytotoxicity effect and was found to have significant antioxidant potential. From the findings of the present study it was concluded that the methanol extract of *Alkanna tinctoria* have various antioxidant, anti-diabetic and phytotoxic activities. Furthermore, analysis is needed to investigate its mechanism of pharmacological activities.

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

1. Introduction

Billions years ago, molecular oxygen was not found in the atmosphere and was introduced by the photosynthetic organisms as O_2 in our environment. Reactive oxygen species (ROS) appeared as uninvited companions with the origination of aerobic life [1]. The photosynthetic byproduct O_2 is a free radical, as it has two impaired electrons of same spin quantum number. This quality makes O_2 to accept electrons from other molecules which lead to formation of group termed as ROS which proved to be very harmful for cells. During cell metabolic path ways, ROS are also continuously produced as byproducts in cell active organelles such as mitochondria, chloroplast and peroxisomes [2-3]. In higher plants and algae the thylakoid membrane of chloroplast, O_2 is continuously produced in the process of photosynthesis. In normal condition, the ROS molecules produced are scavenged by defense mechanisms comprise by various antioxidants [4]. But certain factors are involved in unbalancing of this equilibrium in between the scavenging of ROS and its production. These include various biotic and abiotic stresses such as pollution, salinity, drought, UV radiation, heavy metals, temperature, nutrient deficiency, herbicides and pathogen attacks lead to sudden increase in the ROS level in cell causing significant damage to cell structure and function. According to an estimate, 1-2% of O_2 is enough for the formation of ROS in cell [5-6].

The production of free oxygen molecule O_2 leads to the formation of other ROS like H_2O_2, and OH. In plants, high concentration may result in the minimum production of crop [7-9]. ROS can also damage larger molecules as nucleic acids and oxidizing proteins [10]. The site of production of very reactive ROS also plays a very significant role in its negative response as they can absorb a very short distance in body. These reactive oxygen species can create spontaneous DNA damage in a cell [11] and can also generate response in an inactive gene and make it functional. The effect of stress induced ROS can be controlled by a variety of enzymatic scavengers present in a body as APX, GST, SOD, CAT and GPX and also by non-enzymatic contents such as GSH, ASH, flavonoids, carotenoids and a-tocopherol [12]. All the above enzymatic and non-enzymatic antioxidants are almost present in all cell organelles to mask the ROS negative effects such as systemic signaling, pathogen defense, biotic and abiotic stress-response [13].

Reactive species (ROS and RNS) are produced in human body due to continuous exposure to various categories of reactive agents that results in the production of oxidative dysfunction. Researchers are seeking different ways for strengthening of organism's cellular antioxidant machinery against various stresses to control the harmful effects of reactive molecules [14].

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 are available on the therapeutic uses of medicinal plants. Among these medicinal plants, *Alkanna tinctoria* Tausch, "alkanet", is a Mediterranean plant more knowledge by the use of its roots as red dye, however now is under exploration as having under exploration as having numerous beneficial uses [15]. Now a 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 [16]. Traditionally the plant *Alkanna tinctoria* has been used for the treatment of various human diseases. The present study is therefore arranged to investigate the phytochemical screening and pharmacological activities viz; DPPH, H_2O_2, ABTS and Phytotoxicity of *Alkanna tinctoria* roots extract.

2.Materials and Methods

2.1 Plant collection and Extract preparation

Alkanna tinctoria roots were collected from the nearby area, University of Science and Technology Bannu, KPK, Pakistan. The plants were recognized by their local names and then validated by Dr. Fizan Ullah, Department of Plant Sciences, University of Science and Technology Bannu, A voucher specimen with Accession No. 345 (*A. tinctoria*) was deposited at the Herbarium of University. Extract were prepared according to Sahreen, Khan, Khan (2001) that were then dried and stored at 4°C for further study.

2.2 DPPH Scavenging activity

The DPPH activity was performed following the method of Bibi *et al*., [17]. For preparation of DPPH solution 5g of methanol 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 as a standard.

Scavenging effect % = [(1-absorbance of sample) / control absorbance] x100

2.3 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*., [18] method. Two 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.

H_2O_2 Scavenging activity %= [(1-absorbance of sample) / control absorbance] x100

2.4 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 the standard protocol of Luis et al., [2]. 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 mixed 0.2 ml sample with 0.8 ml 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.

ABTS Scavenging effect %= [(1-absorbance of sample) / control absorbance] x100

2.5 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. 0.1 mg (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 tartrate 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:

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

2.6 . Phytotoxicity assay

For determination of Phytotoxicity assay Mclaughlin and Rogers, [19] was used. The allelopathy/ phyto lethal activities were run in 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 1000 µl/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 dishes they were soaked in water for one hour. Seeds were placed at equal position. Petri plates were incubated at 25°C. The 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.

3 Results and Discussion

3.1 DPPH scavenging free radicals effect

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. The result obtained from different concentrations of methanolic extract of *Alkanna tinctoria* showed significant DPPH free radicals scavenging effect. The findings revealed that methanol extract significantly scavenged the free radicals as was shown by ascorbic acid which has been used as a positive control. The previous literature of antioxidant activity of *Coscinium blumeanum, Fibraurea tinctoria and Arcangelisia flava* fully supports our research findings [20]. Our previous results also in coherent to the present findings [21].

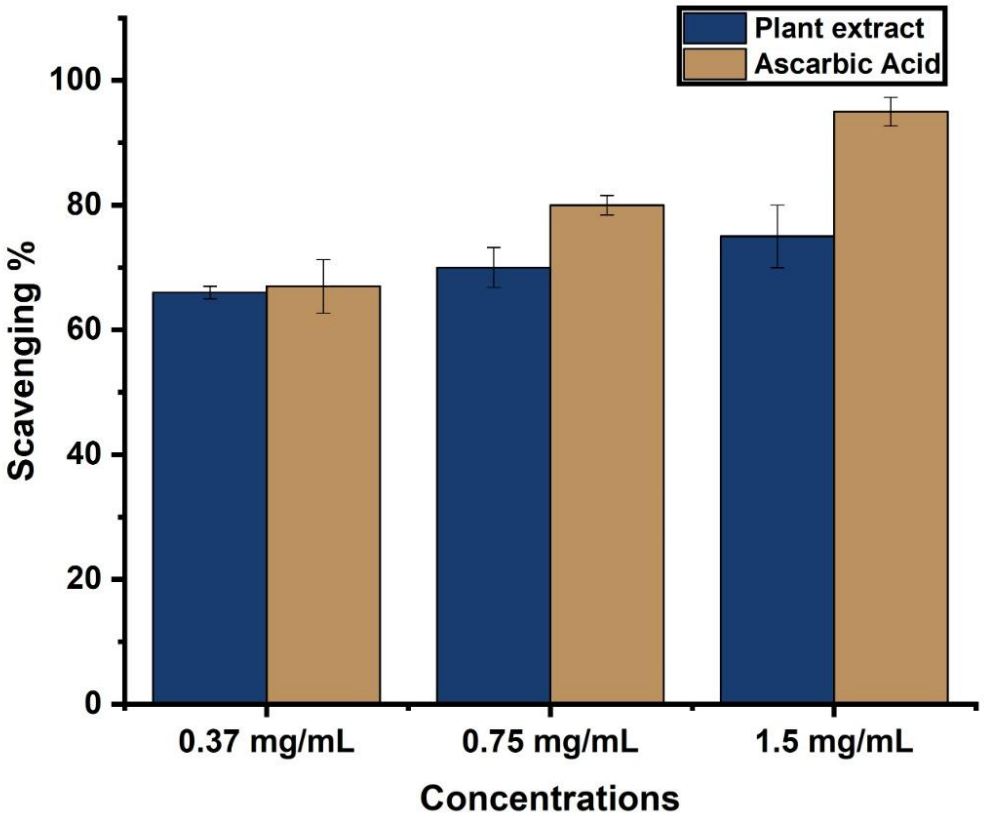

Figure 1. DPPH Scavenging effect of *Alkanna tinctoria* roots extract.

3.2 **Hydrogen peroxide (H_2O_2) activity**

Hydrogen peroxide activity was carried out with different dilution of *Alkanna tinctoria*. All the concentrations of extract of plant of *A. tinctoria* showed significant scavenging effect in the reduction of hydrogen peroxide into water (Figure 2). Similar hydrogen peroxide scavenging activity was reported by the other scientists [22].

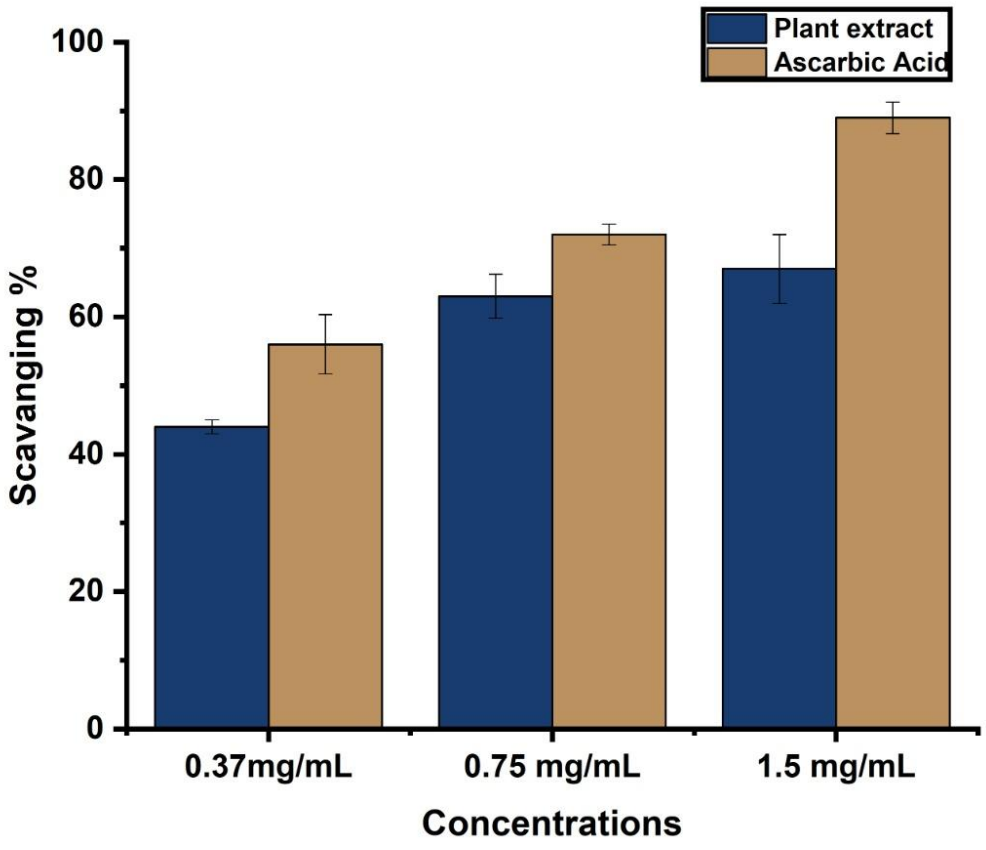

Figure 2. Hydrogen peroxide scavenging ability of *Alkanna tinctoria* roots extract.

3.3 ABTS free radicals scavenging activity

Plant extracts exhibit antioxidant properties and is therefore rich sources of natural antioxidants. 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 (Figure 3). This results accord well with Popovic *et al*. [23] and Ciz *et al.* [24].

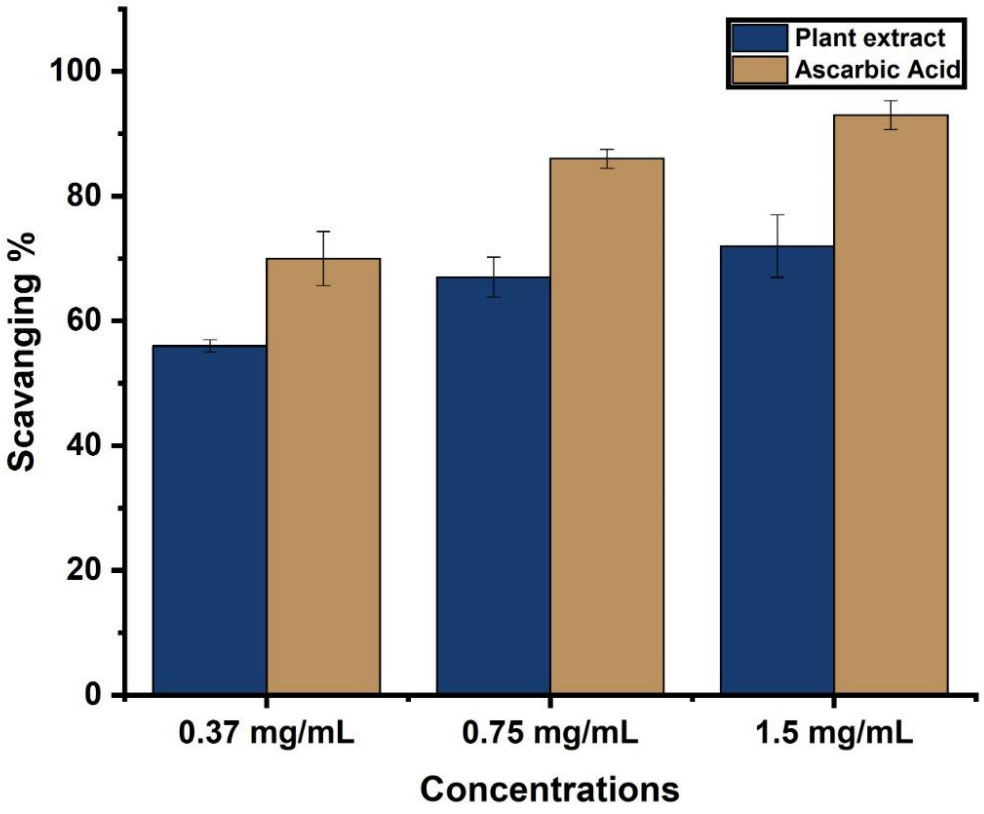

Figure 3. ABTS scavenging ability of *Alkanna tinctoria* roots extract.

3.4 Alpha amylase activity

Alpha amylase activity was performed for the determination of in-vitro anti-diabetic activity of *Alkanna tinctoria* plant extract. Significant inhibition and antidiabetic activity was found in plant extract as a compared to control ascorbic acid (Figure 4). In-vitro-amylase inhibitory activity of plant extract was also determined by Malik et al., [25] revealed similar findings as the present study reported and is coherent to our study.

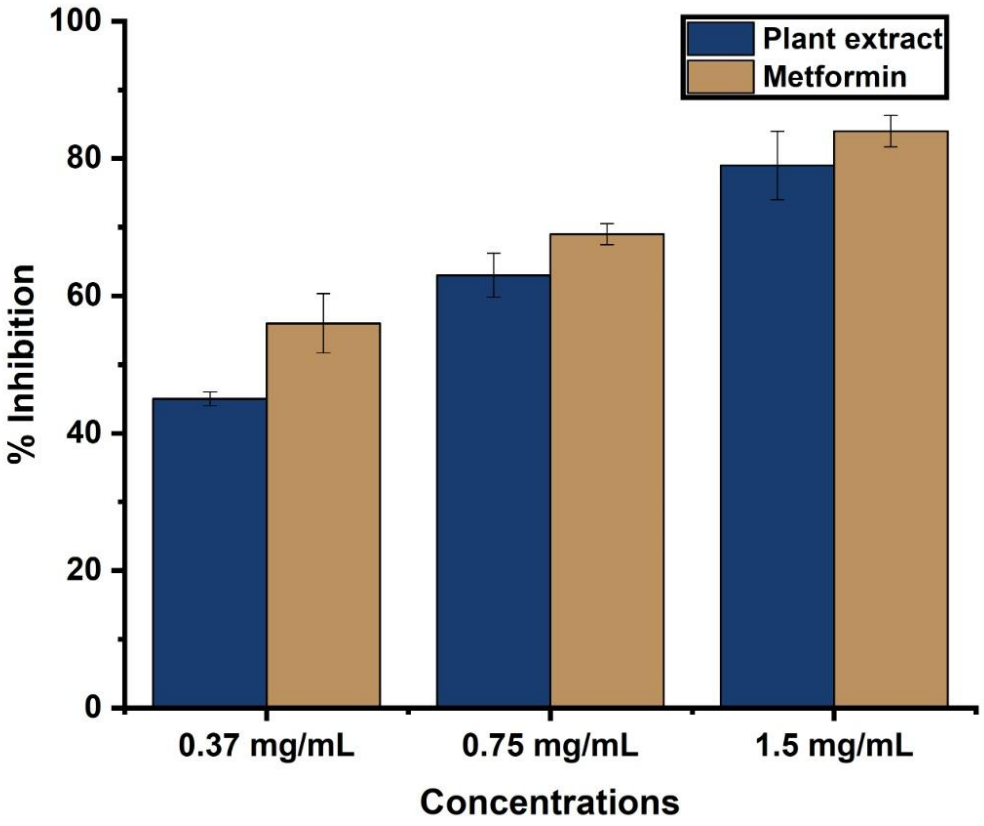

Figure 4. Alpha amylase % inhibition of *Alkanna tinctoria* roots extract

3.5 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. tinctoria* extract was dissolved in different methanol concentrations. Result was obtained at 3 and 7 days of the experiment of roots and shoots were measured as and were showed that there is inversely relation between growth and dose concentrations of extracts in the roots and shoots of the plant (Table 1). Similar results are obtained from other investigation [26].

Table 1. Phytotoxicity assay of *Alkanna tinctoria* roots extract

Concentration	**Roots growth**		**Shoot growth**	
	3 days	**7 days**	**3 days**	**7 days**
100µl/mg	**4.2±0.5**	**6.2±0.6**	**3.9±0.8**	**4.8±0.3**
250µl/mg	**3.3±0.2**	**5.0±0.3**	**2.3±0.5**	**4.0±0.5**
500µl/mg	**3.0±0.3**	**4.5±0.4**	**1.6±0.3**	**2.8±0.4**
1000µl/mg	**2.7±0.1**	**3.0±0.2**	**0.9±0.2**	**1.5±0.2**

Conclusions

The plant under study was selected to perform Antioxidant DPPH, Antioxidant ABTS, Hydrogen peroxide (H_2O_2) Alpha- amylase activity and Phytotoxicity activity. The summary of the data achieved showed that methanol crude extract of plant showed good antioxidant activity: DPPH, ABTS, Hydrogen peroxide (H_2O_2) activity. Significant inhibition and anti-diabetic 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.

References

[1]. Halliwell, B. (2006). Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiology*, *141*(2), 312-322.

[2] Luis, A., Sandalio, L. M., Corpas, F. J., Palma, J. M., & Barroso, J. B. (2006). Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. *Plant Physiology*, *141*(2), 330-335.

[3] Navrot, N., Rouhier, N., Gelhaye, E., & Jacquot, J. P. (2007). Reactive oxygen species generation and antioxidant systems in plant mitochondria. *Physiologia Plantarum*, *129*(1), 185-195

[4] Pfannschmidt, T. (2003). Chloroplast redox signals: how photosynthesis controls its own genes. *Trends in plant science*, *8*(1), 33-41.

[5] Foyer, C. H., & Noctor, G. (2005). Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *The Plant Cell*, *17*(7), 1866-1875.

[6] Bhattacharjee, S. (2005). Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transducation in plants. *Current Science*, 1113-1121.

[7] Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in plant science*, *7*(9), 405-410.

[8] Mahajan, S., & Tuteja, N. (2005). Cold, salinity and drought stresses: an overview. *Archives of Biochemistry and Biophysics*, *444*(2), 139-158.

[9] Tuteja, N. (2007). Mechanisms of high salinity tolerance in plants. *In Methods in enzymology* (Vol. 428, pp. 419-438). Academic Press.

[10] Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, *48*(12), 909-930.

[11] Singh, J., & Thakur, J. K. (2018). Photosynthesis and abiotic stress in plants. In *Biotic and abiotic stress tolerance in plants* (pp. 27-46). Springer, Singapore.

[12] Gill, S. S., Khan, N. A., Anjum, N. A., & Tuteja, N. (2011). Amelioration of cadmium stress in crop plants by nutrients management: morphological, physiological and biochemical aspects. *Plant Stress*, *5*(1), 1-23.

[13] Gratão, P. L., Polle, A., Lea, P. J., & Azevedo, R. A. (2005). Making the life of heavy metal-stressed plants a little easier. *Functional Plant Biology*, *32*(6), 481-494.

[14] Apel, K., & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review in Plant Biology*, *55*, 373-399.

[15] Ahvazi, M., Khalighi-Sigaroodi, F., Charkhchiyan, M.M., Mojab, F., Mozaffarian, V.A. and Zakeri, H., 2012. Introduction of medicinal plants species with the most traditional usage in Alamut region. *Iranian journal of pharmaceutical research: IJPR*, *11*(1), p.185.

[16] Khan, U.A., Rahman, H., Qasim, M., Hussain, A., Azizllah, A., Murad, W., Khan, Z., Anees, M. and Adnan, M., 2015. Alkanna tinctoria leaves extracts: a prospective remedy against multidrug resistant human pathogenic bacteria. *BMC complementary and alternative medicine*, *15*(1), p.127.

[17] Bibi Y, Nisa S, Chaudhary MF, Zia M (2011). Antibacterial activity of some selected medicinal plants of Pakistan. BMC Comp.Alt. Med. 11: 52.

[18] Wasiullah, W., Saleem, J.A.N., Shad, A., Basit, A. and Ullah, F., 2019. Phytochemical Investigation And Pharmacological Activities Of *Heliotropium curassavicum* Linn. *Latin American Applied Research-An international journal*, *49*(2), pp.105-109.

[19] McLaughlin, J.L., and Rogers L.L. (1998); The use of Biological assays to evaluate Botanicals. Drug Interm. J. 32: 513 – 524.

[20] Keawpradub N, Dej-adisai S, Yuenyongsawad S (2005). Antioxidant and cytotoxic activities of Thai medicinal plants named Khaminkhruea: Arcangelisia flava, Coscinium blumeanum and Fibraurea tinctoria. *Songklanakarin J. Sci. Technol.*, 27(Suppl 2): 455-467.

[21] Khan RA, Khan FU, Ahmad M, Shah AS, Khan NS, Khan MR, Shah MS (2010). Phytotoxic and antibacterial assays of crude methanolic extract of *Mentha longifolia* (Linn.) Afri. J. Pharm. Pharmacol. 4: 175-200.

[22] Wettasinghe M, Shahidi F (2000) Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. Food Chem 70:17–26

[23] Popovic, M., B. Kaurinovic, S. Triviv, N. Mimica-Dujic and M Bursac. Effect of alcery Apium graeolens) extracts on some biochemical parametersb of oxidative Stress in mice treated with carbon tetra chloride Phytother, 20, 531-536 (2006).

[24] Ciz, M., H.C. Ova, P. Denev, A. Slavov and A. Lojek omparison of the antioxidant properties of vegetable, Food Cont., 21, 518-523 (2010).

[25] Malik, S., S. Bhushan, M. Sharma, and P. S. Ahuja. 2016. Biotechnological approaches to the production of shikonins: A critical review with recent updates. *Critical Reviews in Biotechnology* 36: 327–340.

[26] Tuteja, N. (2009). Cold, salinity, and drought stress. *Plant stress biology: from genomics to systems biology*, 137-159.