



**INVESTIGATING THE ANTIOXIDANT CHARACTERISTICS, ANTIBACTERIAL
AND ANTIFUNGAL ACTIVITIES OF DATURA STRAMONIUM LEAF**

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ABSTRACT

This study aims at investigating the antioxidant characteristics, antibacterial and antifungal activities of *Datura Stramonium* leaf in Behshahr City in 2014. Free radicals cause numerous diseases in humans. Neutralizing the free radicals, the antioxidants reduce the risk of catching the cardiovascular diseases and stroke, and also prevent the progress of cancer. After preparing and drying the leaves, the extraction is performed by Soxhlet extractor. The antioxidant effect of extracts is investigated by DPPH free radical scavenging methods and measurement of phenol and total flavonoid by spectrophotometry. The antibacterial effect of extracts is also assessed on 4 strains of bacterium including two gram-positive bacteria, namely, *Staphylococcus aureus* PTCC 1112 and *Bacillus serrus* PTCC 502, two gram-negative bacteria, namely, *Escherichia coli* PTCC 1330 and *Pseudomonasa aeruginosa* PTCC 1310 and two fungal strains, namely, *Candida albicans* PTCC 5027 and *Aspergillus fumigates* PTCC 5009 by disk diffusion and well methods. The results indicate that the highest free radical scavenging of leaf extract is seen at the concentration of 250 µg/ml (53.82±0.59) and the rate of phenolic compound equal to 147.33±5.03 mg of Gallic acid per gram and the rate of flavonoid equal to 74.33±5.68 mg of Quercetin per gram. In antimicrobial activity of plant sample by disk diffusion and well methods, the microorganism of *Bacillus cereus* allocated the highest diameter of inhibition zone halo in concentration of 50 and 100 ml. Furthermore, *Candida albicans* species showed a little activity in antifungal activity. The results indicate that the *Datura stramonium* leaf extract is full of phenol

and flavonoid compounds and has high antioxidant properties. The antibacterial activity for leaf extract of diameter of inhibition zone halo is increased by enhancing the concentration.

Keywords: Datura stramonium, antioxidant, antibacterial

INTRODUCTION

The plants are the rich sources of phenolic and Flavonoid compounds and are among the natural antioxidants. The antioxidants reduce the risk of cardiovascular diseases and stroke, and on the other hand prevent the cancer progression which damages the DNA [9], Despite the existence of various antioxidants in plasma, the immune system cannot alone eliminate the free radicals in the body; therefore, it needs to supply the antioxidants from external sources through food sources [8], The secondary metabolites are derived from the plants such as phenol and total flavonoid derived from the plants with strong potential for free radical scavenging, and they are in all different parts of plant such as leaf, fruit, seed, root and skin (3). *Datura stramonium* is a grass and one-year plant from the family of Solanacea. The plant has different heights which seriously depend on the ecological habitat conditions, and usually vary from 20 to 150 cm and reach 200 cm in wet areas. The root is straight with a length of 10-20 cm with numerous white branches. The *Datura stramonium* leaves are bright green and alternate, and long with a length of 10-

15 cm, and the width of 8-10. Its flower is single, large, funnel-shaped, white or cream with a length of 4-6 cm and 5 short and sharp teeth. The stamen is white or purple with a length of 4.5 mm. The first flowers appear in the mid-June and remain on the plant until the beginning of cold season. *Datura stramonium* fruit is a flesh capsule, locular, with external disks, and a part of floral tube remain below it in inverted mode. The fruit has a length of 4- 4.5 cm and more or less oval or spherical. *Datura stramonium* is originated from the tropical regions and is present in all warm and temperate regions of the world, the coasts of Caspian Sea and India. (Berkovb, 2002; Aniszewski, 2007) This plant is distributed in Azerbaijan, Gilan, Talesh (Astara), Mazandaran (Tonekabon), Gorgan, Arak, Kerman, Tehran, Karaj, Firuzabad, Baluchestan, Bandar-e Gaz, Khorasan (near Birjand) and Sistan [9], *Datura stramonium* contains toxic and important alkaloids such as Hyoscyamine, Atropine, Hyoscine, Scopolamine, and also the extractable fixed oil including Datoric acid, Oleic acid and Linoleic acid. (Ahmad, 2009)

MATERIALS AND METHODS

This study is an experimental study on *Datura stramonium* leaves. *Datura* plant is gathered from Behshahr city (Zagh Marz village) and dried in the shade under a gentle air flow and prepared as the powder. The target species is identified by systematic Doctor in herbarium of Islamic Azad University of Tonekabon, and then the soxhlet extraction is done by methanol and water solvent from 30 to 40 grams of powdered plant sample. The ready extract is utilized for testing the activity of non-enzymatic antioxidant (phenol and flavonoid) (Ahmed et al, 2006).

1) Measurement of total phenolic content: This measurement is performed by Folin–Ciocalteu method (1:10 ratio). 5.0 ml of extract with 5.0 ml Folin–Ciocalteu Solution (1:10 ratio) is diluted by distilled water, and then 4 ml of Aqueous sodium carbonate solution (Na_2CO_3) 1 M is added after 5 minutes. The absorption of mixture is read by Spectrophotometer in the presence of Blank at a wavelength of 760 nm after 15 minutes. Gallic acid is utilized as a standard for drawing the calibration curve. The amount of total phenol is reported based on the amount of "mg of Gallic acid per gram of extract". (Ebrahimzadeh et al, 2008)

2) Measurement of total flavonoid content: After measuring 0.5 gram of plant, 10 ml of acidic ethanol is added and centrifuged for a period of 10 minutes. Afterwards, the tubes containing the extract are put in water bath for 10 minutes and finally the absorbance of samples is read in the presence of blank at the wavelength of 415 nm by spectrophotometer. Quercetin is utilized as the standard for drawing the calibration curve. (Ebrahimzadeh, Kheiri, 2008). The amount of total flavonoid is reported based on the amount of "mg of Quercetin per gram of extract".

3) Antioxidant effect: The antioxidant effect of extracts is measured by Brand-Williams method and through DPPH compound (2,2-Diphenyl-1-picrylhydrazyl), so that first 3.9 ml of DPPH solution is added to 1.0 ml of sample solution (mother extract) and then absorption is measured at a wavelength of 515 nm. Afterward, the resulting solution is put in a dark room at the room temperature for 30 minutes, and finally the absorption of samples is measured at a wavelength of 517 nm in the presence of blank sample (methanol). DPPH radical scavenging is calculated according to the formula by Brand-Williams et al (1995).

4) Extraction for doing the microbial testing: Soaking with a little change is done for

preparing this extract. Therefore, the dried powder of plant (50 g) is poured into the Erlenmeyer flask and then 100 ml of solvent (methanol 80%) is then poured on it. The Erlenmeyer flask is put on Shaker for 48-72 hours until the separation of ingredients is well done (Markham, 1982). Afterwards, the obtained mixture is filtered through paper and centrifuged at 5000 rpm for 15 minutes. The obtained extract is put in rotary device to remove the solvent. This extract is utilized for bacterial susceptibility testing (Parekh & Chanda, 2007).

5) Testing the bacterial susceptibility to extracts: The microorganisms used in this study included two gram-positive bacteria, namely, *Staphylococcus aureus* PTCC 1112 and *Bacillus serrus* PTCC 502, two gram-negative bacteria, namely, *Escherichia coli* PTCC 1330 and *Pseudomonasa aeruginosa* PTCC 1310 and two fungal strains, namely, *Candida albicans* PTCC 5027 and *Aspergillus fumigates* PTCC 5009 developed from the laboratory of Microbiology, Islamic Azad University of Tonekabon.

6) Preparing the microbial solution: 24 hours before the test, first the microbial strains (which are prepared from the Laboratory of Microbiology at Islamic Azad University of Tonekabon) are cultured on agar medium in

order to be activated. Afterwards, 5-10 specified colonies from pure culture of bacteria are solved in 1-2 ml of sterile physiological, and a uniform solution prepared. To determine the microbial concentration and its standardization, we should compare it with McFarland suspension by spectrophotometer at the wavelength of 625.

7) Susceptibility testing of bacteria and fungi to extracts using disk diffusion and well method: For this purpose, we separately enter 10, 20, 30, 40, and 50 lambdas from dilution of each extract in blank disk of 6 mm l and wait until the extract is absorbed into the paper blank disc (Dayal, 2001). In well method, we create five wells at the certain and same distance in culture plate and sterilize the bottom of well with melt medium and pour 60, 70, 80, 90, and 100 lambdas of extract in it. Afterwards, we wait until the extract penetrates the medium and then incubate it. The incubation temperature is 24 hours for bacteria at 25 °C and the Mueller-Hinton agar is the medium needed for bacteria and Sabouraud Dextrose agar for fungi (Du saliva, 2009). Gentamicin standard antibiotic disc is utilized as the control.

The ANOVA, descriptions of data, and drawing the diagrams by SPSS and Excel

software are utilized for statistical analysis and mean comparison in independent communities.

RESULTS

All measurements are repeated three times for sample plant and considered significant at the probability levels of 5% and 1%. The results of measurement tests on flavonoid and phenolic compounds (Table 1), the free radical activity by DPPH (Table 2) and the diameter of inhibition zone halo around the disk and the plant extract well, and control of antibiotics (in mm) in the culture of micro-organism (Table 3) are as follows. The results indicate that the highest free radical scavenging of leaf extract is seen at the concentration of 250 $\mu\text{g/ml}$ with mean of 53.82 ± 0.59 and the rate of phenolic compound equal to 147.33 ± 5.03 mg of Gallic acid per gram and the rate of

flavonoid equal to 74.33 ± 5.68 mg of Quercetin per gram. The results of analysis of variance on data in DPPH antioxidant test indicate the significant correlation at the level of 5%. Due to the increase in extract concentration, the radical scavenging is done with high power. In antimicrobial activity by disk diffusion, the maximum diameter of inhibition zone halo is reported at the concentration of 50 ml in the presence of *Bacillus serrus* microorganism with the mean of 30.33 mm. Furthermore, in antimicrobial activity by well method, the maximum diameter of inhibition zone halo is reported at the concentration of 100 ml in the presence of *Bacillus cerrus* microorganism with the mean of 35.33 mm. The effect of *Candida albicans* species is very little and it is reported equal to zero for *Aspergillus fumigates* strain.

Table 1: The total phenol and flavonoid (in milligram per liter) for methanol extract of *Datura stramonium* leaf

Tests	Methanol extract of <i>Datura stramonium</i> leaf
Total Phenol	147.3333 ± 5.03
Flavonoids	74.3333 ± 5.68

Table 2: The values of DPPH free radical scavenging (in percent) by methanol extract of *Datura stramonium* leaf

Concentration (milligrams per liter)	Free radical scavenging (%)
100	51.8267 ± 2.28 a
150	52.2633 ± 1.97 a
200	53.3867 ± 0.44 a
250	53.8267 ± 0.59 a

The similar letters refer to the insignificant values. The comparison test is not done for attributes in which the relevant F test is insignificant.

Table 3: diameter of inhibition zone halo around the disk and well of plant extract and control of antibiotic (in millimeters) in culture of microorganisms

Testing methods	Extract	Concentration (microliter)	<i>Staphylococcus aureus</i>	<i>Bacillus serrus</i>	<i>Escherichia coli</i>	<i>Pseudomonasa aeruginosa</i>	<i>Candida albicans</i>	<i>Aspergillus</i>
Disk	Datura stramonium	10	18.67	22.33	0.00	0.00	0.00	0.00
		20	22.00	22.67	0.00	0.00	0.00	0.00
		30	19.00	25.00	0.00	8.67	0.00	0.00
		40	20.67	28.33	8.67	15.67	0.00	0.00
		50	22.00	30.33	11.00	16.67	7.33	0.00
	Gentamicin	10						
Well	Datura stramonium	60	20.33	21.67	10.33	14.33	0.00	0.00
		70	21.67	24.00	11.67	18.67	0.00	0.00
		80	23.33	27.67	15.67	21.33	0.00	0.00
		90	27.33	32.00	20.33	22.33	7.33	0.00
		100	30.33	35.33	21.67	25.00	7.33	0.00
	Gentamicin	10						

CONCLUSION

The proper use of medicinal plants requires the accurate and scientific data as well as understanding their chemical compounds because the existence of chemical compounds leads to the therapeutic effect in plant[5] This research confirms the existence of numerous phenolic and flavonoid compounds. In fact, the antioxidant activity is directly correlated with the rate of flavonoid and phenolic compounds.

Vidya Shinde and D.A. Dhale investigated the antifungal properties of Datura stramonium extract against the pathogenic fungi in vegetables, and the ethanol extract of stem skin showed the lowest antifungal activity[6].

Jamshidi et al have investigated the methanol extract of several native plants in Mazandaran in terms of flavonoid and phenol rates and found that there is an appropriate correlation between the

antioxidant activity and phenolic compounds [2].

Warda S. AbdelGadir et al (2008) have studied different types of toxic alkaloids in Datura stramonium and reported atropine, hyoscine alkaloids, etc[7].

Eftekhar et al (2005) have found that the methanol extract of aerial organ in Datura species in Gram-positive bacteria has the effect depending on the day, but it has negligible or zero effect on E.Coli and PS bacteria[1].

All alkaloids have natural origins, and the plants from Solanaceae family have high alkaloid content. These alkaloids are available in all parts of these plants including the roots, stems, leaves, flowers, fruits and seeds. Due to the important chemical compounds such as glycol-alkaloids and phenolic compounds, Datura stramonium species has long been utilized as the medicinal species. Confirming the earlier

studies, this study indicates that *Datura stramonium* has the significant antioxidant activity and antibacterial effects. Since the alkaloid compounds are increased in humid climatic conditions, the comparative study of samples from other habitats in the north of country can be useful in explaining the sustainable program for cultivation of these plants.

REFERENCES

1. Eftekhari, F.; Yousefzadi, M., Tafakori, V.F. (2005). Antimicrobial activity of *Datura innoxia* and *Datura stramonium*. *Jan*, 76(1): 118-20.
2. Jamshidi M, Ahmadi HR, Rezazadeh Sh, Fathi F, Mazanderani M.; Study on phenolic and antioxidant activity of some selected plant of Mazandaran province. *Medic Plan* 2010; 9(34) 177-183. [In Persian]
3. Mirzaei A, Mohammadi J, Mirzaei N, Mirzaei M. The Antioxidant Capacities and Total Phenolic Contents Some Medicinal Plant in Iran. *Medical Sciences, Fasa, Iran; Des* 2011; Vol.3, No.1, pp. 104-111.
4. Noguchi N, Niki E. Phenolic antioxidants: A rationale for design and evaluation of novel antioxidant drug for atherosclerosis. *Free Rad Biol Med*. 2000; 28(10):1538-1546.
5. Shariatifar N, Kamkar A, Shams Ardekani M, Misaghi A, Jamshidi A. H, Jahed Khaniki Gh. Quantitative and Qualitative Study of Phenolic Compounds and Antioxidant Activity of Plant *Pulicaria Gnaphalodes*. *Ofogh-e-Danesh. GMUHS Journal*. 2012; Vol.18, No. 1.
6. Vidya Shinde and D.A. Dhale. Antifungal properties of extracts of *Ocimum tenuiflorum* and *Datura stramonium* against some vegetable pathogenic fungi. *Journal of Phytology* 2011, 3(12): 41-44.
7. Warda S. AbdelGadir, Hayfa a M.A. Ishag, Amel, O. Bakhiet. Toxicity of *Datura stramonium*, *Capsicum frutescens* and their mixture to Bovine Chicks. *Sud.J. Vet.Sci.Anim.Hush*. 2008; Vol.47.
8. Young IS, Woodside J. Antioxidants in health and disease. *J Clin Pathol*. 2001; 54: 176-186.
9. Zargari A. *Medicinal Plants*. University of Tehran publication 1991, [in Persian].