IJBPAS, October, 2015, 4(10), Special Issue: 762-768

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

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

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ABSTRACT

This study aims at investigating the antioxidant characteristics, antibacterial and antifungal activities of Datura stramonium stem in Behshahr City in 2014. The plant extracts are from the good sources of antimicrobial compounds and are highly treatable. After preparing and drying the stem of studied plant, extracting is done by Soxhlet extractor. The antioxidant effect of extract is investigated by DPPH free radical scavenging and assessment of total flavonoid and phenolics by spectrophotometry. The antimicrobial effect of stem extract on 4 strains of bacteria and the antifungal effect of extract on 2 fungal strains are investigated by disk diffusion and well methods. The results indicate that the highest free radical scavenging of stem extract is seen at the concentration of 250µg/ml with the mean of 31.26± 0.62 and the rate of phenolic compound equal to 97.66±4.93 mg of Gallic acid per gram and the rate of flavonoid in stem extract equal to 33.66±1.52 mg of Quercetin per gram. In antimicrobial activity of plant sample by disk diffusion, the microorganism of Bacillus cereus allocated the highest diameter of inhibition zone halo in concentration of 100 ml. The fungal activity of stem methanol extract is also reported equal to zero. The results indicate that the antioxidant activity is directly correlated with flavonoid and phenolic compound. Furthermore, the increased diameter in antibacterial activity will lead to the enhanced diameter of inhibition zone halo.

Keywords: Datura stramonium, antioxidant, anti-bacterial activity, anti-fungal activity

INTRODUTION

In terms of weather conditions and geographical situation in the field of grown medicinal plants, Iran is one of the best places in the world and the plants are widely used for treating as the traditional medicine. The study on the medicinal plants of treatment in different regions of Iran and investigation of their experimental and clinical characteristics are among the important measures in this regard. (1, 2) The plants are the rich sources of flavonoid and phenolic compounds and are among the natural antioxidants. On the one hand, the antioxidants reduce the risk of cardiovascular disease and stroke, and on the other hand, will prevent the cancer development which damages the DNA. (3) Datura is a herbaceous and one-year plant from the family of Solanacea with the scientific name of Datura Steramoniom and local name of Guzmasel, Totauleh, Bagham, Talanour, NAfir, Bache Ghir, Diren, Joze MAsel, Kajla, Marge Moushe, Sineh Azeghi, Jozmash, evil candy, and devil's weed. The plant height varies and is highly dependent on the ecological conditions of habitat. Datura stramonium has the origin in tropical and warm temperate regions and in all moderate and warm regions of the world, the coasts of the Caspian Sea and India[2], [4].This plant is grown in Azerbaijan, Gilan, Talesh (Astara), Mazandaran (Tonekabon), Gorgan, Arak, Kerman, Tehran, Firuzabad, Baluchistan, Bandar Gaz, Khorasan (near Birjand), and Sistan [4].Datura contains the toxic and important alkaloids such as hyoscyamine, atropine, hyoscine, Scopolamine, and also the extractable fixed oil including Datoric acid, Oleic acid and Linoleic acid[1].

MATERIALS AND METHODS

This study is an experimental study on Datura stramonium stem. 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 of Folin–Ciocalteu Solution (1:10 ratio) is diluted by distilled water, and then 4 ml of Aqueous sodium carbonate solution (Na2co3) 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 prepared from the laboratory of Microbiology, Islamic Azad University of Tonekabon.

6) Preparing the microbial solution: 24 hours before the test, the microbial strains (which are prepared from the Laboratory of

Microbiology at Islamic Azad University of Tonekabon) are first 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 Ċ 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 are presented in the following tables1-3.

The results indicate that the rate of phenolic compound in stem is equal to 97.66±4.93 mg of Gallic acid per gram and the rate of flavonoid in stem equal to 33.66±1.52 mg of Quercetin per gram. Furthermore, the highest free radical scavenging of stem extract is seen at the concentration of 250µg/l with the mean of 31.26±0.62. The Mean comparison indicates the significant effect of concentration on the free radical scavenging, and the significant difference in concentration of 250 compared to other concentrations. The increase in concentration of antioxidant activity is done with high power.

In antimicrobial activity by disk diffusion, the maximum diameter of inhibition zone halo is reported in the concentration of 50 ml in the presence of Bacillus serrus microorganism with the mean of 23.67 mm. Furthermore, in antimicrobial activity by well method, the maximum diameter of

inhibition zone halo is reported in the concentration of 100 ml in the presence of *Pseudomonasa aeruginosa* microorganism with the mean of 20.67 mm. The effects of *Candida albicans* species and *Aspergillus fumigates* strain are reported equal to zero in this research.

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

Tests	Methanol extract of Datura stramonium stem
Total Phenol	97.6667 ± 4.93
Flavonoid	33.6667 ± 1.52

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

Concentration (milligrams per liter)	Free radical scavenging (%)
100	19.0267± 0.37 c
150	20.76667± 1.17 bc
200	22.5900± 2.65 b
250	31.2667± 0.62 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.

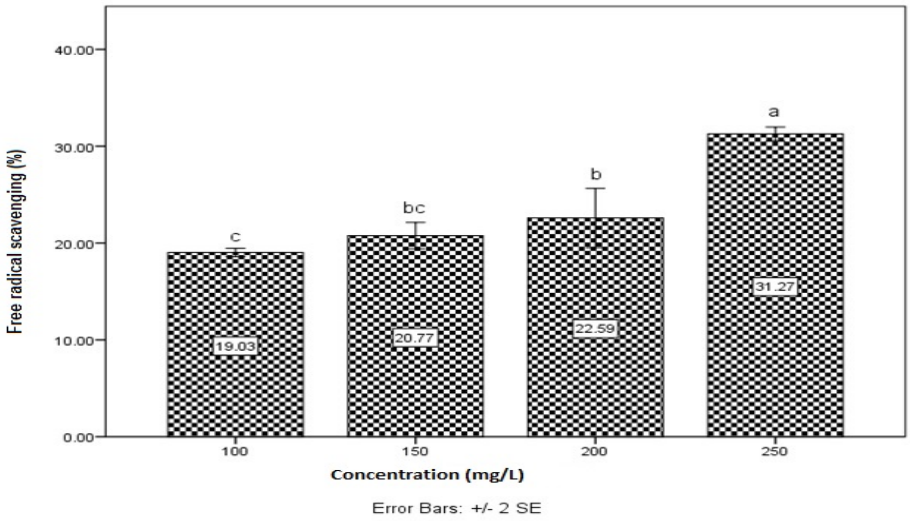

Figure 1: Changes of free radical in different concentrations

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)	Staphylococcus aureus	Bacillus serrus	Escherichia coli	Pseudomonasa aeruginosa	Candida albicans	Aspergillus
Disk	Datura stramonium	10	10.33	11.67	0.00	0.00	0.00	0.00
		20	12.33	14.67	0.00	0.00	0.00	0.00
		30	15.67	18.67	0.00	0.00	0.00	0.00
		40	20.33	20.67	0.00	8.53	0.00	0.00
		50	21.67	23.67	9.33	10.67	0.00	0.00
	Gentamicin	10						
Well	Datura stramonium	60	8.33	12.33	0.00	9.67	0.00	0.00
		70	10.33	14.33	0.00	11.33	0.00	0.00
		80	12.33	14.00	0.00	14.67	0.00	0.00
		90	12.33	16.67	0.00	14.67	0.00	0.00
		100	14.00	19.67	11.33	20.67	0.00	0.00
	Gentamicin	10						

CONCLUSION

This study aimed at measuring the antioxidant and antibacterial power of Datura stramonium stem. The phenolic compounds play major roles in antioxidant activity. Primarily, the increased in total phenolic compounds will lead to the enhanced antioxidant property. The phenolic compounds with high molecular weight (tannins) have a great ability to remove the free radicals and this ability is more dependent on the number of aromatic rings and the nature of replacing groups of hydroxyl[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 [4].

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 has the day-depending effect in Gram-positive bacteria, but it has negligible or zero effect on E.Coli and P.S bacteria [2].

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. Confirming the earlier studies, this study indicates that Datura stramonium has the significant antioxidant activity and antibacterial effects.

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