IJPSR (2015), Vol. 6, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 December, 2014; received in revised form, 27 January, 2015; accepted, 21 May, 2015; published 01 July, 2015

PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF METHANOLIC EXTRACT OF *DATURA STRAMONIUM* SEEDS

Samier A. Waza , Prashant Anthony and Sikander Dar *

Department of Chemistry, Sam Higginbottom Institute of Agriculture, Technology and Sciences (Formerly AAI-DU), Allahabad, U.P. India

Keywords:

Datura stramonium, Phytochemical screening, Methanolic extract, IC_{50}, Zone of inhibition.

Correspondence to Author:
Sikander Dar

PhD research scholar
Department of chemistry Sam Higginbottom, Institute of Agriculture, Technology and Sciences (Formerly AAI-DU), Allahabad, U.P. India-211007

E-mail: sikanderdar786@gmail.com

ABSTRACT: The present study was concerned with phytochemical screening of methanolic extract of *Datura stramonium* seeds and its antioxidant and antimicrobial activities. The methanolic extract was prepared by using Soxhlet apparatus. The result of phytochemical screening of methanolic extract of *Datura stramonium* seeds shows presence of various phytoconstituents like alkaloids, flavonoids, amino acids, tannins, saponins, carbohydrates and terpenoids. The methanolic extract reduced the concentration of DPPH free radical with an efficacy near to that of standard antioxidant Gallic acid, but less than BHT. The IC_{50} value for methanolic extract was 0.82mg/ml. The Nitric oxide activity of methanolic extract was also close to the Gallic acid. The IC_{50} value obtained for the methanolic extract was 0.70mg/ml. The antibacterial activity was detected by agar well diffusion method against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Staphyloceceus aureus.* The zones of inhibitions obtained were recorded and analyzed against standard control of Ampicillin. The methanolic extract showed higher antibacterial activity of 24.0 mm against *E.coli* and least antibacterial activity of 13mm against *P.aeruginosa.* The present study suggests that the use of seeds of this plant may be exploited for health supplements.

INTRODUCTION: Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates terpenoids, steroids and flavonoids [1]. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(7).3021-26

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(7).3021-26

They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [2]. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms *in vitro*[3].

Datura stramonium contains different type of phytochemical including Saponins, Tannins, Steroids, Alkaloids, Flavonoids, Phenols and Glycosides [4]**.** Among *Solanaceae* plants, *Datura stramonium* L. is highly regarded by the workers, since it has a great resource of tropane alkaloids. Botanical alkaloids are one of the important botanical products and form the major part of medicinal compounds. Tropane alkaloids (i.e. atropine, hyoscyamine and scopolamine) are

generally found in *Hyosyamous*, *Datura stramonium* L. and *Belladonna*. The chemical investigations of Datura demonstrated that leaves and seeds especially were rich in alkaloids, including atropine, hyoscyamine, and scopolamine. These compounds are included in many official pharmacopoeias because of their anticholinergic activities. Recently, several pharmacological investigations have been conducted on Datura. In fact, different extracts obtained from this plant have been reported to exhibit antimicrobial [5] and antifungal activities [6] as well as hypoglycemic[7] and antimutagenic properties [8]. Thus, the aim of this study is Phytochemical analysis, Antioxidant and Antimicrobial Activities of Methanolic Extract of *Datura stramonium* Seeds

MATERIAL AND METHODS:

Sample preparation:

The dried seeds of *Datura stramonium* which were obtained from seed pods of Datura plant. These dried seeds were pulverized using sterile laboratory mortar and pestle to obtain the powdered form. These were stored in airtight glass containers protected from sunlight until required for analysis**.** 20g dried powder of *Datura stramonium* plant seeds were weighed and transferred into conical flask for extract preparation as given below.

Preparation of extract:

The dried and powdered seeds were extracted with methanol using soxhlet extractor for 24h at a temperature not exceeding the boiling point of the Solvent [9]. The extracts were filtered and then concentrated to dryness, transferred to glass vials and kept at 4º C before use.

Phytochemical analysis:

The methanolic extract was tested for the presence of various bio-active compounds by using following tests[10,11,12].

Test for alkaloids:

Mayor‘s test: 2 ml filtrate was mixed with 1% HCl and about 6 drops of Mayor’s reagent was added. A Creamish or pale yellow precipitate appeared which indicated the presence of respective alkaloids.

Test for flavonoids:

By magnesium ribbon: 2 ml filtrate was added to concentrated HCl and magnesium ribbon was put into the solution. Pink-tomato red color indicated the presence of flavonoids.

Test for amino acids:

Ninhydrin test: 1 ml of the extract was treated with few drops of Ninhydrin reagent. Purple color indicated the presence of amino acids.

Test for tannins:

Ferric chloride test: 1 ml of the extract was treated with few drops of 0.1% ferric chloride. Brownish green colour indicated the presence of tannins.

Test for saponins:

Froth test: 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was add and boiled for 5 min. The mixture then filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Test for steroids:

Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. If red colour produces in the lower chloroform layer indicates the presence of steroids.

Test for carbohydrates:

Fehling’s test: Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of carbohydrates.

Test for terpenoids:

Salkowski test: 5 ml of extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Test for Chalcones: By using Ammonium hydroxide: 2 ml of Ammonium hydroxide when added to 0.5g methanolic extract. Appearance of reddish color indicates the presence of chalcones

Antioxidant Activity Assay:

DPPH Free Radical Scavenging Assay:

The DPPH radical scavenging activity assay elucidated by[13] was followed. The hydrogen atom or electron donating ability of the corresponding methanolic extract was measured from the bleaching of the purple coloured methanol solution of DPPH. This spectrophotometric assay uses the stable radical, 2,2- diphenylpicrylhydrazyl (DPPH), as a reagent. The working solution of the test extract was prepared in methanol. BHT and Gallic acid was used as standard in 1-100µg/ml solution. DPPH (0.002%) was prepared in methanol and 1ml of sample solution separately. Their solution mixtures were kept in dark for 30min and absorbance was measured at 517nm. DPPH solution (1ml) was used as blank. The absorbance was recorded and % inhibition was calculated using the formula given below.

$$\% \text{ Scavenging activity} = \frac{A-B}{A} \times 100$$

Where

A= absorbance of the blank and

B= absorbance of the sample

Scavenging method:

The Nitric Oxide scavenging method elucidated by [14] was followed**.** Nitric oxide (NO) was generated from Sodium Nitroprusside (SNP) and was measured by Griess reagent. SNP in aqueous solution at physiological pH spontaneously generates. NO which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess Reagent. Scavenging of NO compete with oxygen leading to reduced production of NO. SNP (10mM) in phosphate buffer saline (PBS) was mixed with different concentration of extract (100-1000µg/ml) of the drug dissolved in methanol and incubated at 25^0C for 180 min.

The samples from the above were reacted with Griess reagent (1% sulphanilic acid, 0.1% naphthylamine and 3% phosphoric acid). The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilic acid and subsequent coupling with napthylamine was recorded at 546 nm and referred to the absorbance of gallic acid and BHT, used as positive controls treated in the same way with Griess reagent.

Anti-bacterial activity Assay:

Test organisms:

The organisms used comprises of two gram-positive bacteria (*S. aureus* and *B. subtilis*) and two gram-negative bacteria (*E. coli* and *P. aeruginosa*). The test organisms were obtained from the Research Laboratory of Microbiology and Fermentation Technology SHIATS, Allahabad, India.

Agar well diffusion method:

Agar well diffusion method elucidated by[15] was followed. The antibacterial activity of crude methanolic extract of *Datura stramonium* seeds against four pathogenic bacteria was evaluated by using agar well diffusion method. The Nutrient agar plates were prepared by pouring 15 ml of molten media into sterile petri-plates. About (10^8-10^9) colony- forming unit per ml were used. Wells or cups of 5mm size were made with sterile borer into agar plates containing the bacterial inoculum. 10µL of microbial broth was spread on the surface of nutrient agar plates, 20µL volume of the sample extract of concentration (2mg/ml) was poured into a well of inoculated plates. Ampicillin (2mg/ml) was used as a positive control which was introduced into the well instead of plant extract.

Solvent methanol was used as a negative control which was introduced into well instead of plant extract. The plates thus prepared were kept at room temperature for ten minutes allowing the diffusion of the extract into the agar [16]. After incubation for 24 hrs at 37^0C, the plates were observed. The antibacterial activity was present on the plates; it was indicated by an inhibition zone surrounding the well containing the plant extract. The zone of inhibition was measured and expressed in millimeters. Antibacterial activity was recorded when radius of zone of inhibition was greater than 4mm [17]. The antibacterial activity results was considered as inactive if < 4.5 mm; 4.5-6mm as partially active ; while 6.5-9 mm as active and greater than 9mm as very active [18].

RESULTS AND DISCUSSION:

The phytochemical screening of methanolic extract of *Datura stramonium* seeds was performed by using various tests and the results obtained are shown in **Table 1** given below:

TABLE 1: PHYTOCONSTITUENTS OF SEEDS OF *DATURA STRAMONIUM*

Name of phytoconstituents	Name of test	Result
Alkaloids	Mayor's test	+ve
Flavonoids	By magnesium ribbon	+ve
Amino acids	Ninhydrin test	+ve
Tannins	Ferric chloride test	+ve
Saponins	Froth test	+ve
Steroids	By using sulphuric acid	-ve
Carbohydrates:	Fehling's test	+ve
Terpenoids	Salkowski test	+ve
Chalcones	By using ammonium hydroxide	-ve

Antioxidant activity of *Datura stramonium* seed extract:

The DPPH free radical scavenging activity of methanolic extract of seeds of *Datura stramonium* at various concentrations was determined and compared with that of the standard BHT and Gallic acid **(Table 2 and Fig. 1).** Five different working solutions of methanolic extract were used having concentration (1.0, 0.8, 0.6, 0.4 and 0.2 mg/ml). All the tested samples showed lower DPPH radical scavenging activities (56.07%, 43.67%, 40.31%, 18.48%, and 13.36%) respectively when compared with the standards. The methanolic extract reduced the concentration of DPPH free radical with efficiency near to that of Gallic acid but less than BHT. The IC50 value for methanolic extract was 0.82mg/ml.

TABLE 2: DPPH FREE RADICAL SCAVENGING ASSAY (%) OF METHANOLIC EXTRACT OF *DATURA STRAMONIUM* SEEDS, GALLIC ACID AND BHT.

Conc. (mg/ml)	Extract	%	Gallic acid	%	BHT	%
1.0	0.347±0.011	56.07	0.383±0.011	58.45	0.234±0.018	66.42
0.8	0.445±0.017	43.67	0.410±0.006	55.53	0.265±0.006	61.97
0.6	0.563±0.023	40.31	0.470±0.007	49.02	0.332±0.011	52.36
0.4	0.644±0.046	18.48	0.532±0.028	42.29	0.365±0.007	47.63
0.2	0.644±0.057	13.36	0.554±0.037	39.91	0.445±0.042	36.15

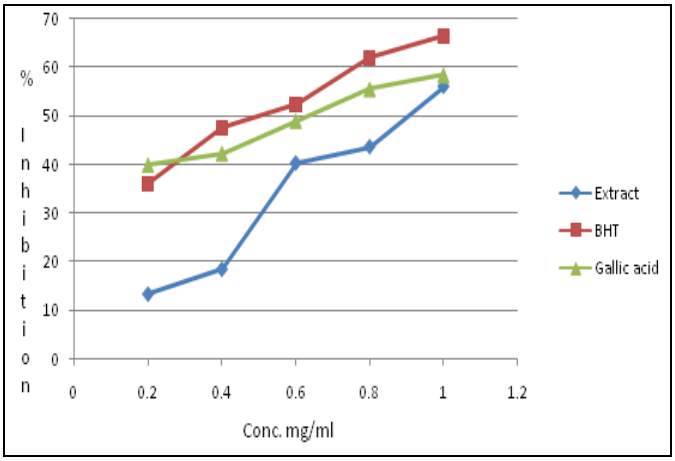


FIG.1: DPPH SCAVENGING ACTIVITY FOR BHT, GALLIC ACID AND METHANOL EXTRACT OF *DATURA STRAMONIUM.*

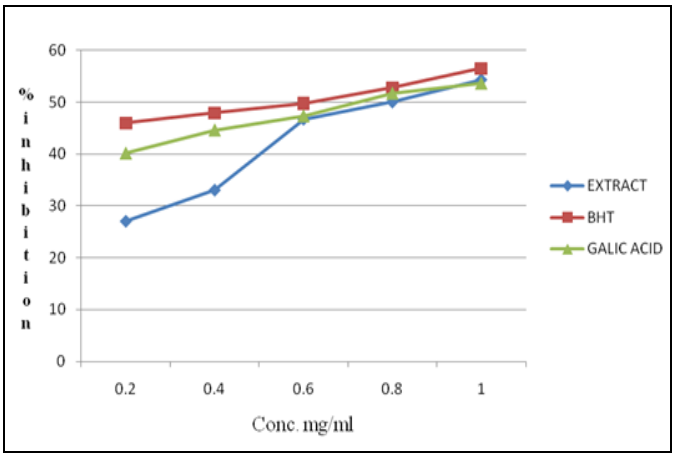


FIG. 2: NITRIC OXIDE SCAVENGING ACTIVITY FOR BHT, GALLIC ACID AND METHANOLIC EXTRACT OF *DATURA STRAMONIUM.*

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which were measured by Griess Reaction. Nitric oxide radical generated from nitroprusside at physiological pH was found to be inhibited by the methanolic extract as shown in the (**Table 3 and Fig. 2).** The IC50 values have been found to be 0.70mg/ml, 0.65mg/ml and 0.60mg/ml for the methanolic extract, gallic acid and BHT respectively.

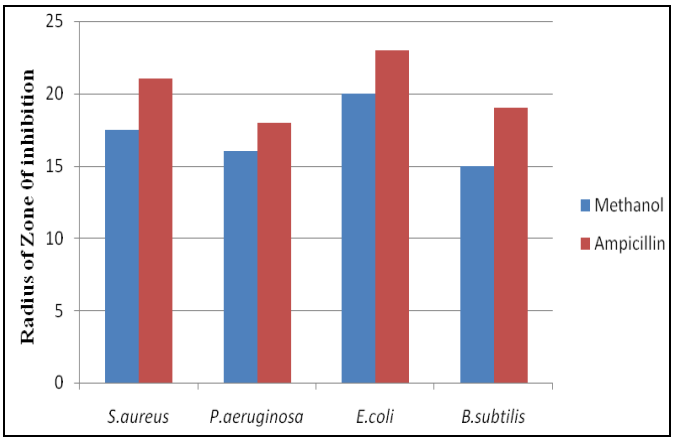
Antibacterial activity:

The antibacterial activity reveals that the methanolic extract of *Datura stramonium* seeds is highly active against both Gram positive and Gram negative bacteria. The methanolic extract shows highest zone of inhibition (20mm) against *E.coli* followed by (17.50mm) zone of inhibition against *S.aureus*, (16mm**)** against *P.aeruginosa* and lowest (15mm) zone of inhibition against *B.subtili*. Graph

1 summarizes the microbial growth of methanolic extract and ampicillin used as a positive control.

TABLE 3: NITRIC OXIDE SCAVENGING METHOD (%) OF METHANOLIC EXTRACT OF *DATURA STRAMONIUM*, GALLIC ACID AND BHT.

Conc. (mg/ml)	Extract	%	Gallic acid	%	BHT	%
1.0	0.512±0.031	54.28	0.539±0.010	53.67	0.698±0.060	56.51
0.8	0.560±0.088	50.00	0.552±0.010	51.74	0.758±0.036	52.77
0.6	0.597±0.072	46.69	0.579±0.005	49.38	0.806±0.016	49.78
0.4	0.750±0.055	33.03	0.633±0.008	44.66	0.837±0.023	47.85
0.2	0.823±0.066	29.70	0.684±0.010	40.20	0.868±0.013	45.91



GRAPH 1: ANTIBACTERIAL ACTIVITY (in mm) OF METHANOLIC EXTRACT AND AMPICILLIN (STANDARD)

CONCLUSION: On the basis of results obtained it can be concluded that *Datura stramonium* seeds possess alkaloids and flavonoids and have potent antioxidant and antibacterial activities. Further the potential of this plant can be explored more and more, in order to develop an alternative therapy for treatment of various diseases. The present study also suggests that the use of this medicinal plant may be exploited for health supplements. Thus justifying its traditional use.

AKNOWLEDGEMENT: The authors sincerely thank the authorities of department of Chemistry and department of Microbiology, SHIATS for their support in completion of the work.

REFERENCES:

1. Edoga HO, Okwu DE and Mbaebie BO: Phytochemicals constituents of some Nigerian medicinal plants. Afr. J. Biotechnol. 2005; 4:685-688.
2. Vasu K, Goud JV, Suryam A and Singara MA: Biomolecular and phytochemical analyses of three aquatic angiosperms. African Journal of Microbiology 2009; 3:418–421.
3. Cowan MM: Plant products as antimicrobial agents. Journal of Clinical Microbiology. 1999; 12:564-582.
4. Shagal MH, Modibbo UU and Liman AB: Pharmacological justification for the ethnomedical use of Datura Stramonium stem-bark extract in treatment of diseases caused by some pathogenic bacteria. International Research of Pharmacy and Pharmacology 2012; 2:016-019.
5. Kagale S, Marimuthu T, Thayumanvan B, Nandakumar R and Samiyappan R: Antimicrobial activity and induction of systemic resistance in rice by leaf extract of Datura metel against Rhizoctonia solani and Xanthomonas oryzae pv Oryzae. Physiology and Molecular Plant Pathology 2004; 65:1–100.
6. Dabur R, Singh H, Chhillar AK, Ali M and Sharma GL: Antifungal potential of Indian medicinal plants. Fitoterapia 2004; 75:389–391.
7. Murthy K, Nammi S, Kota MK, Krishna RV, Koteswara RN and Annapurna A. Evaluation of hypoglycemic and antihyperglycemic effects of Datura metel (Linn.) seeds in normal and alloxan-induced diabetic rats. Journal of Ethnopharmacology 2004; 91(1): 95–98.
8. Reid KA, Maes J, Maes A, Staden J, Kimpe N and Mulholland DA: Study of African medicinal plants. Journal of Ethnopharmacology 2006; 106:44–50.
9. Linn J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jager AK and Staden JV: Preliminary screening of some traditional Zulu materials for anti-inflammatory and anti-microbial activities. Journal of Ethnopharmacology 1999; 68:267-274.
10. Harborne JB: Phytochemical Methods: A guide to modern techniques of plant analysis. 3rd ed. Chapman and Hall, London, 2008; 49-188.
11. Sofowra A: Medicinal Plants And traditional Medicine in Africa. Spectrum Books Ltd., Ibadan Nigeria 1993; 25:191-289.
12. Trease GE, Gajendiran K and Evans WC: Phytochemical screaning of Citrus. Pharmacognosy, 11th edition, Bailliere Tindall, London 1989; 25:45-50.
13. Nooman A, Khalaf A, Al-Othman A, El-Agbar A and Farah H: Antioxidant activity of some common plants. Turkish Journal of Biology 2008; 32:51-55.
14. Balak KN, Ponda AB, Raj NR, Shrivastava A and Prathani R: The evaluation of Nitric Oxide Scavenging Activity of Acalypha indica Linn Root. Asian Journal of Research in Chemistry 2009; 2:4164-4169.
15. Ahmad I and Beg AJ: Atimocrobial and phytochemical studies on 45 Indian medicinal plants against multidrug resistant human pathogens. Journal of Ethnopharmacology 2001; 74:111-123.

16. Rios JL, Recio MC and Villar A: Screening methods for natural products with antimicrobial activity: a review of the literature. Journal of Ethnopharmacology 1988; 23:127-149.
17. Hammer KA, Carson CF and Riley TV: Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology 1999; 86: 985-990.
18. Junior A, Alves TM, Silva A, Brandao AF, Grandi FM, Smania TSM, Smania FA and Zani C: Biological screening of Brazilian medicinal plants. Brazilian Journal of Science 2000; 95:367.

How to cite this article:
Waza SA, Anthony P and Dar S: Phytochemical Analysis, Antioxidant and Antimicrobial Activities of Methanolic Extract of *Datura Stramonium* Seeds. Int J Pharm Sci Res 2015; 6(7): 3021-26.doi: 10.13040/IJPSR.0975-8232.6(7).3021-26.