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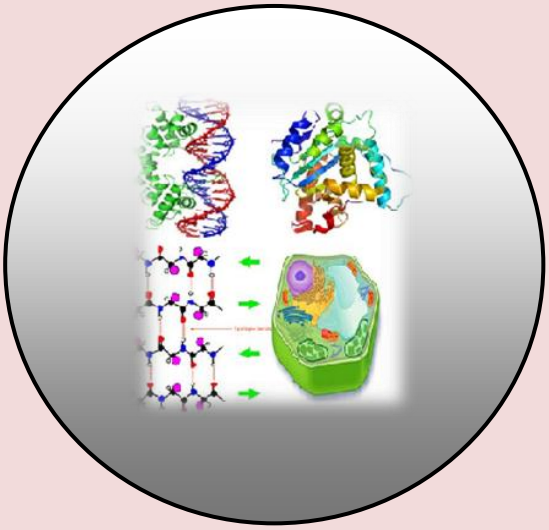
ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 38 (2), 2021 Pages No. 128-139



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 38, No. 2, 128-139, 2021
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 38/02/7475/2021

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

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http:// www.sasjournals.com
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RESEARCH PAPER
Received: 09/08/2021 Revised: 15/08/2021 Accepted: 15/08/2021

Analysis of Antibacterial and Antioxidant Activities of *Datura Stramonium* L.

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ABSTRACT

Medicinal plant Datura stramonium L. was screened for its antibacterial and antioxidant activities by Agar-well diffusion method and DPPH free radical scavenging assay, respectively. Methanol and acetone extracts of different parts (seed and leaf) were taken for the assay. Antibacterial activity of acetone and methanol extracts of plant was tested against four pathogenic bacteria i.e. Escherichia coli, Listeria monocytogenes, Salmonella typhi and Shigella dysenteriae at different concentrations i.e. 25, 50, 75 and 100%. Antioxidant activity was also studied for both extracts of plant at 20, 40, 60, 80 and 100 µg/mL concentrations. Results showed that both the extracts of plant showed significant antibacterial and antioxidant activities. Methanol seed extract of Datura stramonium L. showed maximum zone of inhibition and methanol leaf extract of plant exhibited higher antioxidant capacity with lesser IC_{50} values than other extracts. The present study proved the medicinal value of the selected plant and thus plant extracts can be used for further investigation for gaining their proper therapeutic knowledge.

Key Words: Datura stramonium L., Plant extracts, Agar-well diffusion and DPPH.

INTRODUCTION

Since ancient times, different plants have been used as a source of medicines and play a dominant role in maintenance of human health (Farombi, 2003). A variety of drugs could be obtained from medicinal plants. About 80 % individuals from developing countries rely on plant based preparations used in their traditional medicinal system and as the basic needs for human primary health care (Ellof, 1998). Natural products supply the important sources of main compounds to the pharmaceutical industry and up to 40% of modern drugs are being obtained from natural sources (Jassim and Naji, 2003). Uses of plants in India for medicinal purposes dates back over to 5000 years and has become a major part of Ayurveda, which contains over 8000 herbal remedies (Akerele *et al*., 1991).

Many higher plants have been the source of medicinal agents from very earliest times, and they continue to play a prominent role in the primary health care of around 80% of the world's population (Farnsworth *et al*., 1985). OECD decided to undertake a study of biodiversity issues concerning medicinal plant species (Akerele *et al*., 1991). There are estimations, which indicate that at least 90% of medicinal plants are present in the wild (Anonymous, 1997). The affordability, reliability, availability and low toxicity of medicinal plants in therapeutics made them popular and acceptable by all religions and implementation in health care all over the world (Akharaiyi, 2011).

Human infections predominantly those concerning with microorganisms i.e. bacteria, fungi, viruses and nematodes are the reasons of serious infections in tropical and subtropical countries of the world (Mohanasundari *et al*., 2007). Antimicrobial agents are basically significant in decreasing the global problem of infectious diseases (Bhatia and Narain, 2010). Though, rise and distribution of multidrug resistant (MDR) strains in pathogenic bacteria have developed a significant public health risk as there are fewer, or even sometimes no, effective antimicrobial agents are present for the infections produced by these pathogenic bacteria (Boucher *et al.,* 2009; Giamarellou, 2010). A huge number of medicinal plants have been known as resources of natural antimicrobial compounds as a substitute that can probably be effective in handling these challenging bacterial infections (Iwu *et al*., 1999).

Damages produced by free radicals usually associated to aging and diseases, such as arteriosclerosis, diabetes, cancer, and cirrhosis (Halliwell and Gutteridge, 1984). Antioxidants are substances that interrupt or delay the oxidation of cellular oxidizable substrates and are used to preserve food quality from oxidative worsening of lipids. They exert their properties by scavenging ROS, activating battery-operated detoxifying proteins, or evading the generation of ROS.

MATERIALS AND METHODS

Collection of plant material

The plant was collected from Kundli village of Theog tehsil at an altitude of 2,310 m in Shimla district of Himachal Pradesh and brought to the laboratory for further analysis.

Procurement of Bacteria

Different strains of bacteria (*Shigella dysenteriae, Escherichia coli, Salmonella typhi* and *Listeria monocytogenes*) have been procured from Department of Biotechnology, HPU Shimla for screening antibacterial properties of different plant extracts.

Revival of Pathogen

The collected pathogens were revived in nutrient broth and stored in nutrient agar slants at 4°C.

Maintenance and Preservation of Pure Cultures

Pure cultures of all the bacteria were maintained on nutrient medium broth and preserved in refrigerator. Sub-culturing was done at regular intervals in order to maintain the cultures. Each bacterial species was transferred from parent source to maintain and preserve the cultures.

Processing of Plant Material

The leaf and seed of plant was first washed under tap water and then treated with 2% Mercuric chloride. After that the leaves was cut into smaller pieces and allowed to shade dry for 15-20 days.

After drying leaf and seed were crushed to form a fine powder with the help of pestle mortar. Prepared fine powders were stored at room temperature, in air tight containers.

Preparation of Acetone and Methanol Extracts

Acetone and methanol extracts of dried leaves and seeds were prepared to screen antimicrobial activity. 5 gm of material was taken in Erlenmeyer flask to which 50 ml of required solvents (i.e. methanol and acetone) were added. The flask was covered with aluminium foil and placed at safe place for 3-5 days for extraction. Material was filtered using Whatman filter paper no.1 and the extract was evaporated at 40ºC using rotary evaporator. The extract was collected and weighed. At last, a stock solution of 50 mg/ml conc. was prepared.

Antibacterial Activity Test using Agar-well Diffusion Method

Different extracts (acetone and methanol) of *Datura stramonium* L. were screened using agar-well diffusion method. Nutrient agar medium (Beef extract 1 g, Yeast extract 2 g, Sodium Chloride 1 g, Peptone 5 g, Agar 20 g, Distilled Water 1000 mL) was used throughout the investigation. The medium was autoclaved at 121.6ºC for 30 minutes and poured into Petri plates. Bacteria were grown in nutrient broth for 24 hours. A 100 µL of bacterial suspension was spread on each nutrient agar plate. Agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri plate. The wells in each plate were loaded with 25, 50, 75 and 100% concentration of prepared plant extracts. The Petri plate kept as a control contained pure solvent only. The plates were incubated at 37±2ºC for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all the three replicates and the average values were tabulated (Hemashenpagam and Selvaraj, 2010).

Antioxidant Activity Test by DPPH Radical Scavenging Activity Assay

The free radical scavenging activity of plant extracts was measured using 2, 2 Diphenyl-1-picrylhydrazyl (DPPH) as described by Blois (1958). Briefly, to 1mL of different concentrations (5, 10, 15, 20 and 25 µg/mL) of plant or test extract, 1 mL of DPPH (0.1 mM in methanol) was added. Corresponding blank sample was prepared and ascorbic acid was used as reference standard. Mixture of 1mL methanol and 1 mL DPPH solution (without plant extract) was used as control. All the tests were carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark using UV-VIS spectrophotometer. The percentage of inhibition was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = \left(\frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}}\right) \times 100$$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample.

Graphs were plotted against percent inhibition v/s conc. of plant extracts and standard ascorbic acid in order to find out the values of slope and y-intercepts. IC_{50} value (the amount of antioxidant required to decrease the initial DPPH concentration by 50%) for each extract and ascorbic acid was evaluated using the following equation given below:

$$IC_{50} = \frac{50 - Y\text{-}Intercept}{\text{Slope}}$$

RESULTS

Antibacterial Activity Screening

The antibacterial activity of plant extracts of *Datura stramonium* L. was determined by Agar-well diffusion method against four pathogenic bacteria (*(Listeria monocytogenes, Salmonella typhi, Escherichia coli* and *Shigella dysenteriae).* Streptomycin was taken as a standard and positive control. The results of the above assay are shown in Tables-1.1 to 1.2. and fig. A-D. Maximum zones of inhibition were reported in methanol seed extract of *Datura stramonium* L. against the bacterium *E. coli.*

(A) For *Datura stramonium* L. Seed Extract in Methanol

Table-1.1 shows antibacterial activity of methanol seed extract of *D. stramonium* against four bacteria i.e. *Listeria monocytogenes, Escherichia coli, Salmonella typhi* and *Shigella dysenteriae* at different concentrations (25%, 50%, 75% and 100%). At 25% conc., bacteria produced zones of inhibition i.e. 11.67 mm, 20.33 mm, 10.00 mm and 11.66 mm against *L. monocytogenes, E. coli*, *S. typhi* and *S. dysenteriae* respectively. At 100% concentration, a maximum zone of inhibition was shown against bacterium *E. coli* (28 mm) and minimum zone of inhibition was recorded against *S. typhi* (13.66 mm).

(B) For *Datura stramonium* L. Seed Extract in Acetone

From Table-1.1, it is clear that acetone seed extract of *D. stramonium* L. plant showed zones of inhibition against different testing bacteria at different concentrations. Maximum zone of inhibition was recorded in 100% concentrations (13 mm) against bacterium *E. coli* and minimum inhibition was found against bacterium *Salmonella typhi* (11.7 mm).

(A) For *Datura stramonium* L. Leaf Extract in Methanol

It is clear from the data that methanol extract of leaf showed minimum antibacterial activity against *S. dysenteriae*. With the increase in the conc. of the leaf extract, zones of inhibition also increased. At 100% concentration leaf extract produced zones of inhibition i.e. 13 mm, 20.67 mm, 12.33 mm and 12 mm against the bacterium *L. monocytogenes*, *E. coli, S. typhi* and *S. dysenteriae,* respectively. The maximum antibacterial activity was shown against *E. coli* (20.67 mm) at 100% concentration.

(B) For *Datura stramonium* L. Leaf Extract in Acetone

It is evident from Table-1.2 that acetone extract of leaves of *D. stramonium* gave inhibition diameters of 11.3 mm, 11 mm, 11.3 mm and 11.3 mm against bacteria *L. monocytogenes*, *E. coli, S. typhi* and *S. dysenteriae* respectively. With gradual increase in concentration of acetone leaf extract from 25% to 100% there is also increase in zones of inhibition. At 100% concentration maximum activity was shown against the bacterium *S. typhi* (14.3 mm) and minimum zones of inhibition was shown against *E. coli* (13.7 mm).

To study the antioxidant activity of *Datura stramonium* L.

To study the antioxidant activity of different extracts *of Datura stramonium* L., the DPPH (2, 2- diphenyl-1- picrylhydrazyl) radical scavenging assay was followed. The results of the assay are shown in Tables-2.1 to 2.3 and Figs. A-C.

In this experiment, we have taken ascorbic acid as standard solution and by making different concentrations; we compared the antioxidant activity of given plant extracts. Minimum IC_{50} value was worked out in case of ascorbic acid. The results of antioxidant activity of standard (ascorbic acid) as well as extracts of different parts of the plant are given in Tables-2.1 to 2.3 and Figs. A-C. By comparing the IC_{50} values we can predict the antioxidant activities of different extracts of the plant.

Free radical scavenging activity of ascorbic acid

DPPH (2, 2- diphenyl-1- picrylhydrazyl) radical scavenging activity of ascorbic acid (standard solution) was calculated and results were expressed in percentage inhibition corresponding to the various concentrations of ascorbate. The wavelength for this assay was fixed at 517 nm. The graph was plotted between the concentrations of the standard solution used and percentage inhibition of DPPH. The results are represented in Table-2.1 and Fig. A.

Free radical scavenging activity of *Datura stramonium* L. seeds and leaves extracts

DPPH (2, 2- diphenyl-1- picrylhydrazyl) radical scavenging activity of methanol and acetone extracts of *Datura stramonium* L. seeds and leaves was studied and results showed in % inhibition corresponding to the various concentrations of plant extracts. The graphs were plotted against the concentrations of the extract used and percentage scavenging activity of DPPH free radical. The results are represented in Table-2.2 and Fig.-B for seed extracts in methanol and acetone and in Table-2.3 and Fig.-C for the leaf extracts in methanol and acetone. DPPH radial scavenging activity was found to be highest in methanol extract of *D. stramonium* L. leaves at 100 µg/mL concentrations i.e. 62.556%. IC_{50} values of methanol extracts of seed and leaf were 94.579 µg/mL and 68.553 µg/mL, respectively. The acetone extracts of seed and leaf of *Datura stramonium* L. showed IC_{50} values 124.752 µg/mL and 165.356 µg/ml respectively.

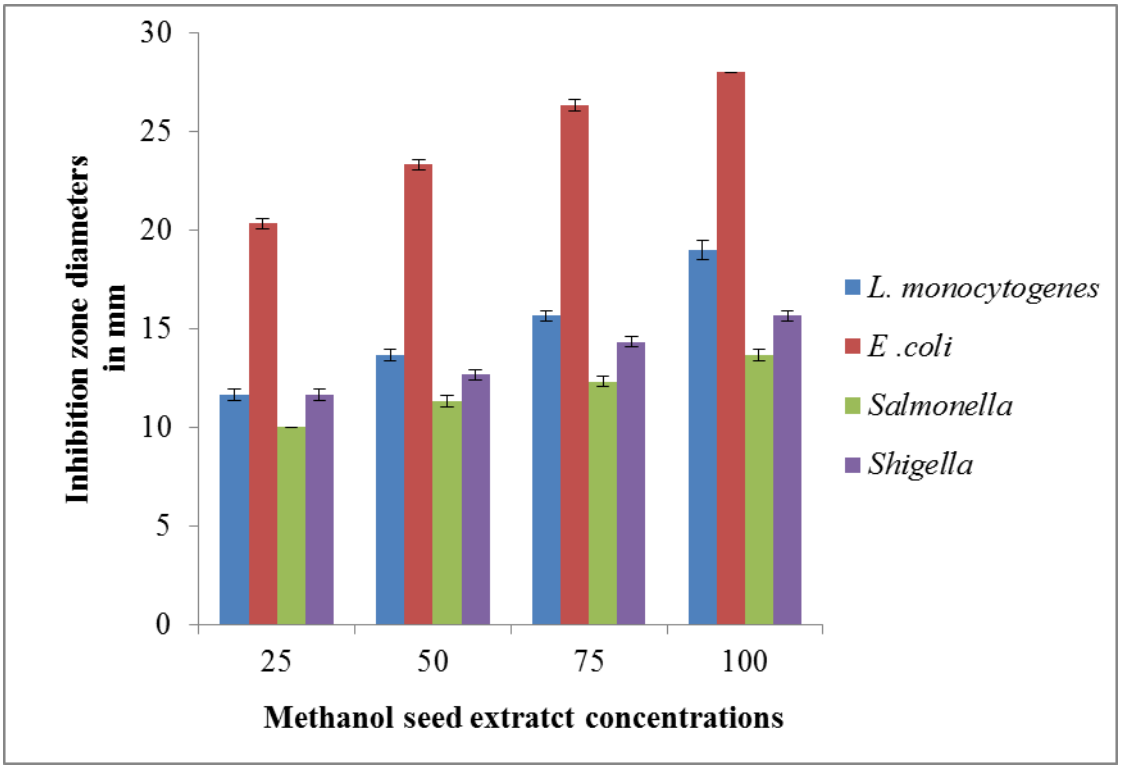

Figure A. Antibacterial activity of Methanol seed extract of *D. stramonium.*

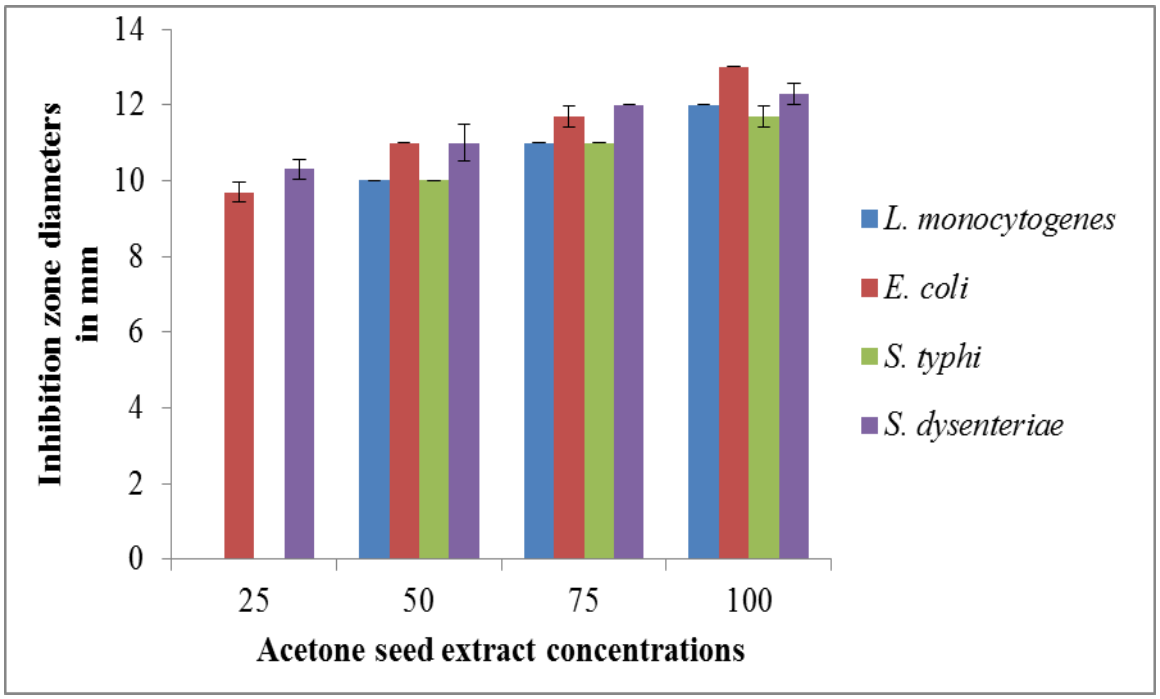

Figure B. Antibacterial activity of Acetone seed extract of *D. stramonium*.

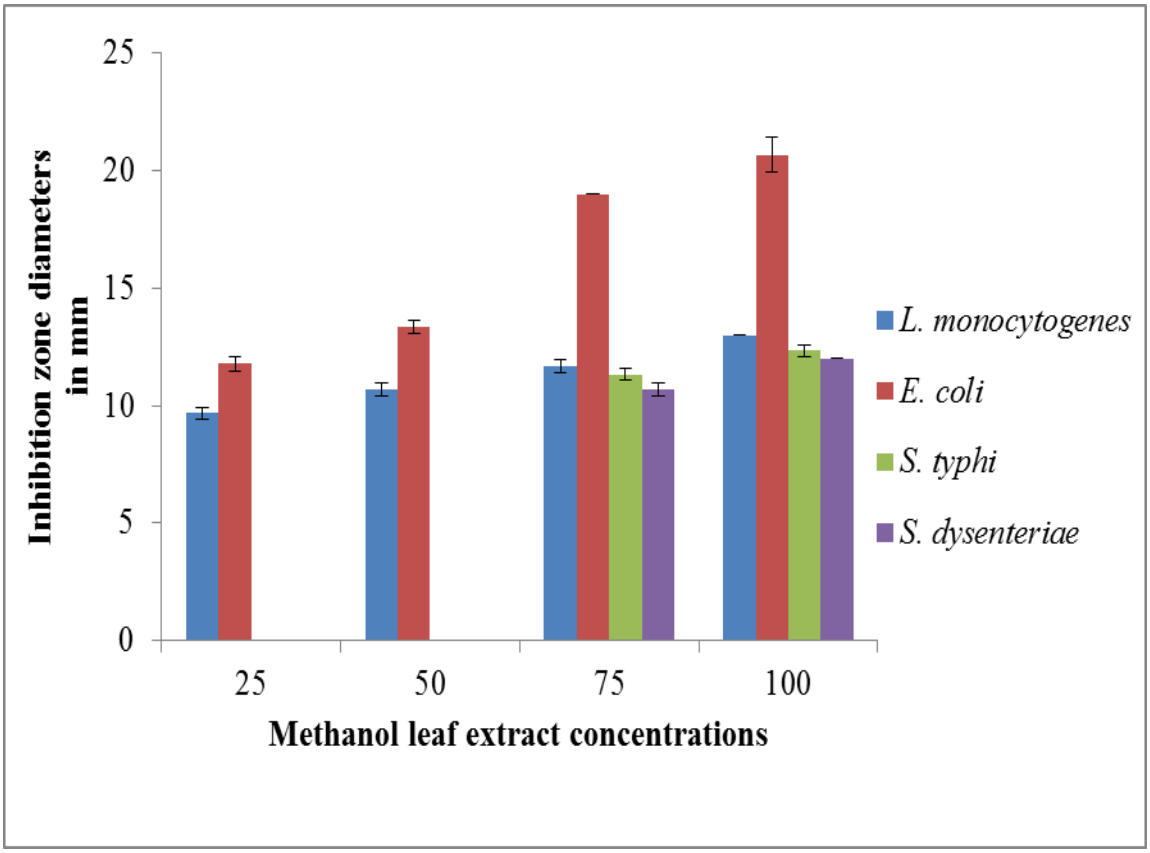

Figure C. Antibacterial activity of Methanol leaf extract of *D. stramonium*.

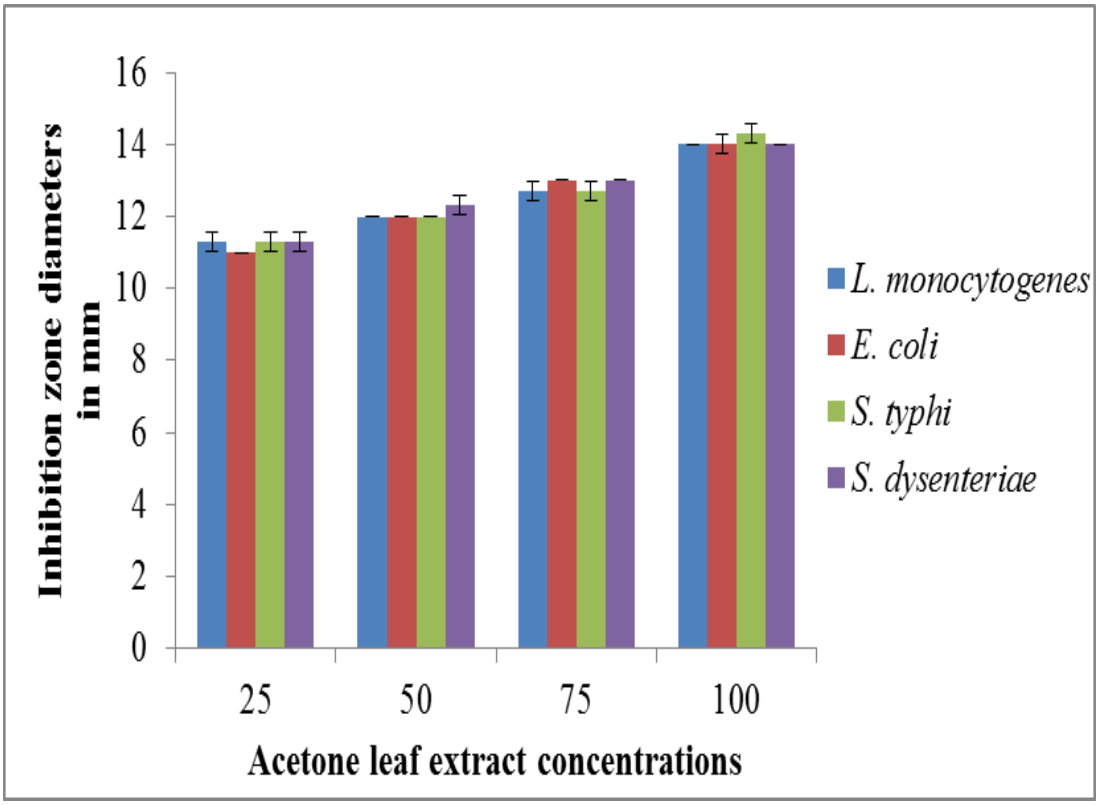

Figure D. Antibacterial activity of Acetone leaf extract of *D. stramonium*.

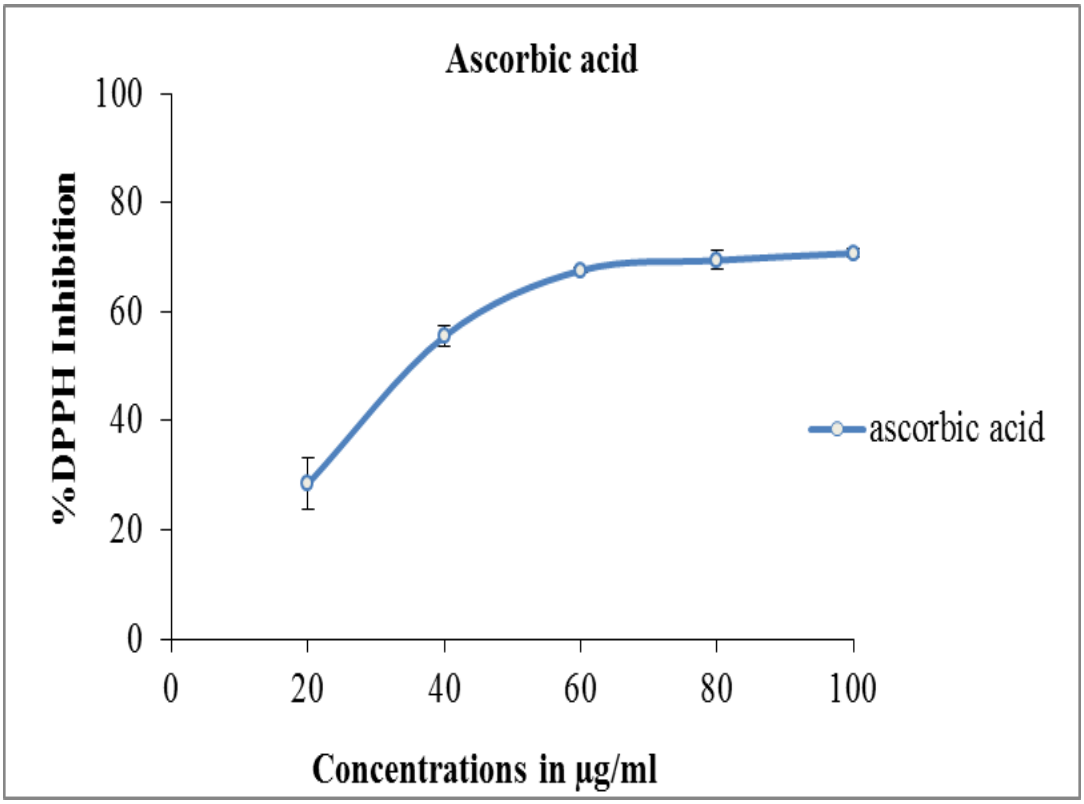

Figure A. Scavenging activity of ascorbic acid at different concentrations.

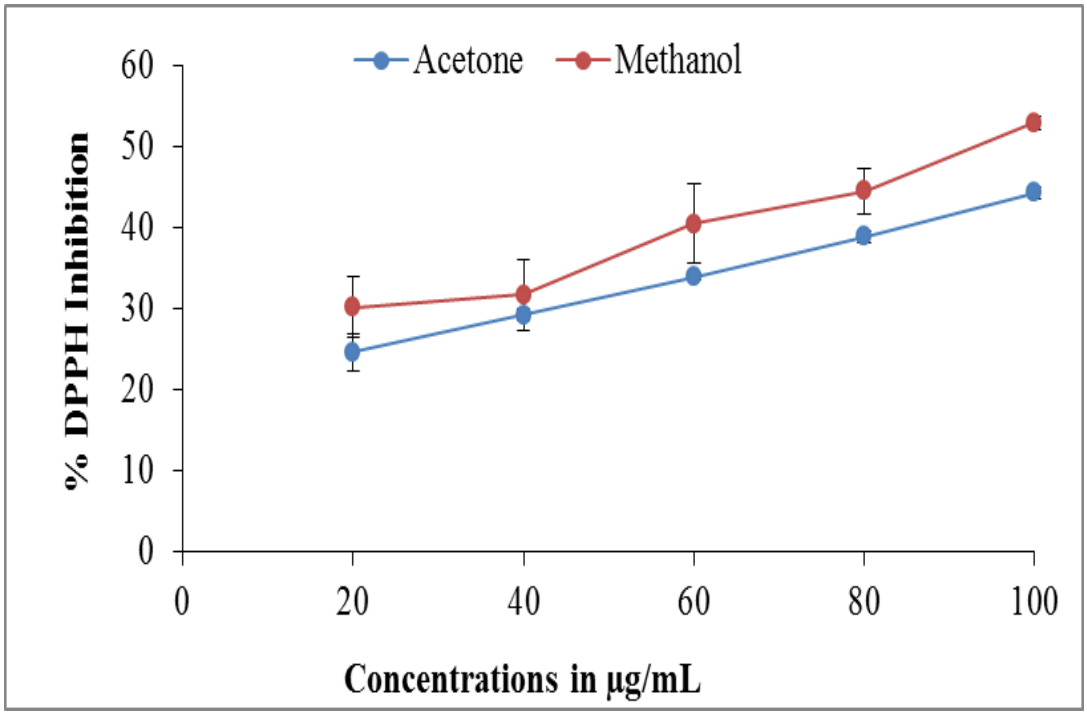

Figure B. Scavenging activity of *Datura stramonium* L. seed extracts at different concentrations in methanol and acetone.

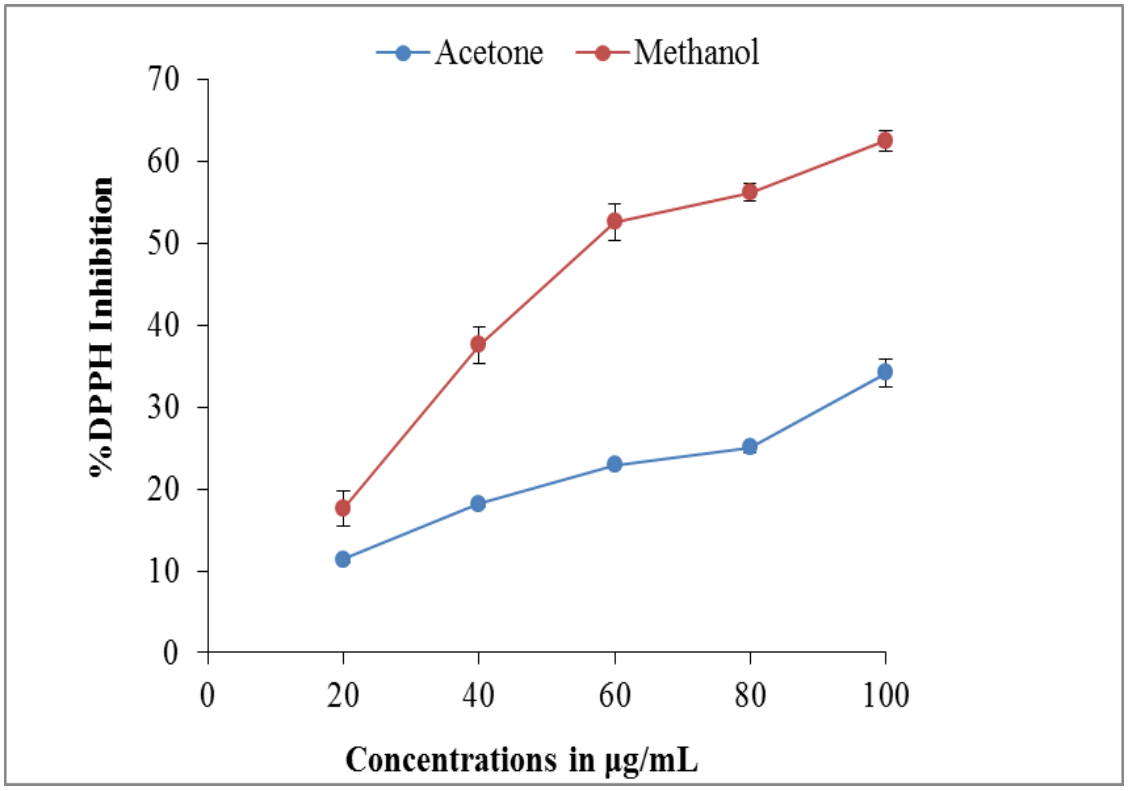

Figure C. Free radical scavenging activity of *Datura stramonium* L. leaf extracts at different concentrations in methanol and acetone.

Table 1.1. Zones of inhibition produced by seed extracts of *Datura stramonium* L. at different concentrations in acetone or methanol.

Extracts	Concentrations (%)	Inhibition zone diameters in mm (±S.E.)			
		L. monocytogenes	***E. coli***	***S. typhi***	***S. dysenteriae***
Methanol Extract	**Control**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	11.67±0.027	20.33±0.47	10.00±0.00	11.66±0.27
	50	13.67±0.027	23.33±0.47	11.33±0.27	12.66±0.27
	75	15.67±0.027	26.33±0.47	12.33±0.27	14.33±0.27
	100	19.00±0.47	**28.00±0.00**	13.66±0.27	15.66±0.27
Acetone Extract	**Control**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	-	9.7±0.27	-	10.3±0.27
	50	10±0.00	11±0.00	10±0.00	11±0.47
	75	11±0.00	11.7±0.27	11±0.00	12±0.00
	100	12±0.00	**13±0.00**	11.7±0.27	12.3±0.27

Each data point's represents mean of three replicates ± S.E. (standard error).

Table 1.2. Zones of inhibition produced by leaf extracts of *Datura stramonium* L. at different concentrations in acetone and methanol.

Extracts	Concentrations (%)	Inhibition zone diameters in mm (± S.E.)			
		L. monocytogenes	***E. coli***	***S. typhi***	***S. dysenteriae***
Methanol Extract	**Control**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	9.66±0.27	11.73±0.29	-	-
	50	10.67±0.27	13.33±0.27	-	-
	75	11.67±0.27	19.00±0.00	11.33±0.27	10.67±0.27
	100	13.00±0.00	**20.67±0.72**	12.33±0.27	12.00±0.00
Acetone Extract	**Control**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	11.3±0.27	11±0.00	11.3±0.27	11.3±0.27
	50	12±0.00	12±0.00	12±0.00	12.3±0.27
	75	12.7±0.27	13±0.00	12.7±0.27	13±0.00
	100	14±0.00	13.7±0.27	**14.3±0.27**	14±0.00

Each data point represents mean of three replicates ± S.E. (standard error).

Table 2.1. Free radical scavenging activity (%) of the control i.e. ascorbic acid at different concentrations.

	Concentrations (µg/mL)	Methanol Extract	IC_{50} Value (µg/mL)
Ascorbic acid **(Control)**	20	28.456	**43.067**
	40	55.487	
	60	67.576	
	80	69.436	
	100	70.738	

Table 2.2. Free radical scavenging activity (%) of the seed extracts of the *Datura stramonium* L. at different concentrations in methanol and acetone.

Name of the plant	Concentrations (µg/mL)	Methanol Extract	IC_{50} Value (µg/mL)	Acetone Extract	IC_{50} Value (µg/mL)
Datura stramonium L. **(Seed)**	20	30.201	**94.579**	24.621	**124.752**
	40	31.704		29.242	
	60	40.476		33.939	
	80	44.486		38.864	
	100	52.882		44.242	

Table 2.3. Free radical scavenging activity (%) of the leaf extracts of the *Datura stramonium* L. at different concentrations in methanol and acetone.

Name of the plant	Concentrations (µg/mL)	Methanol Extract	IC_{50} Value (µg/mL)	Acetone Extract	IC_{50} Value (µg/mL)
Datura stramonium L. **(Leaf)**	20	17.601	**68.553**	11.429	**165.356**
	40	37.668		18.175	
	60	52.691		22.937	
	80	56.278		25.079	
	100	62.556		34.206	

DISCUSSION

Antibacterial Activity Screening

It has been observed that with increase in concentration of leaf and seed extracts, the zones of inhibition also increased. Methanol seed extract shown maximum diameter of zone of inhibition i.e. 28.00 mm against *E. coli* than all other extract, while for its acetone extract maximum diameter of zone of inhibition was 13.00 mm against *E. coli*. In case of leaf extracts maximum zone of inhibition i.e. (20.67 mm) was reported by methanol leaf extract at 100% concentration against bacterium *E. coli.* Antibacterial activities of methanol and acetone extracts of the plant showed positive results against all the bacteria.

Okwu and Igara (2009) studied the antibacterial activity of alkaloid from *Datura metel* Linn. leaves. The antibacterial activity was performed by a filter paper disc diffusion technique and minimum inhibitory concentrations. Their study demonstrates that *D. metel* possess antibacterial activities and justify the traditional use of *D. metel* in phyto-medicine. The compound isolated from *D. metel* can be used by pharmaceutical firms for drug formulation. Hussain *et al*. (2016) investigated the antibacterial activities of methanol extracts of leaves, stems, roots and seeds of *Datura inoxia* against different strains of bacteria following the agar well diffusion method. The bacteria *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Proteus* spp., *Streptococcus pneumoniae* and *Staphylococcus aureus* were more affected but *Pseudomonas aeruginosa* showed resistance to the extracts.

The results of present study are in agreement with the work of earlier workers. However some variations can be attributed to different geographical conditions.

Antioxidant Activity

As per the results, better antioxidant activity was observed in methanol leaf extract (68.553 µg/mL) and least activity was observed in case of acetone leaf extract.

The antioxidant activity of leaf extracts of *Datura stramonium* was studied by Sreenivasa *et al*. (2012) by using the DPPH method. Their results proved that the crude methanol extracts of *Datura stramonium* showed significant antioxidant activity.

The antioxidant property of methanol leaf and seed extracts of *Datura innoxia* and *Datura metel* was studied by Bhardwaj *et al*. (2016). According to them the extract of *D. innoxia* showed higher activity as compared to *D. metel*. Iqbal *et al*. (2017) investigated the antioxidant activities of methanol extracts of seeds of *Datura stramonium*. Their results indicated that the methanol extract of *Datura* seeds showed higher phenolic content and contributes to the higher antioxidant activity.

CONCLUSION

From the performed investigation it was concluded that the selected plant showed considerable activity against all the tested pathogenic bacteria. Maximum diameter of zone of inhibition (28.00 mm) was reported for the methanol seed extract against *E. coli.* In case of antioxidant activity, methanol leaf extract showed stronger activity than the other extracts. This study suggests that the plant extracts possess potent antibacterial and antioxidant activity, which might be helpful in preventing or slowing the progress of various bacterial and oxidative stress-related diseases.

ACKNOWLEDGMENTS

Authors want to put on record their gratitude to the Chairperson, Department of Biosciences, Himachal Pradesh University, Shimla (India) for providing Lab facilities.

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