RESEARCH ARTICLE

Antibacterial Potential of some Plant Extracts against *Escherichia coli*, along with its Phytochemical Analysis, Total Phenolic Content, and Antioxidant Properties

Upma Srivastava, Pooja Singh*

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Abstract

In vitro antibacterial activity of 21 plant methanolic extracts were investigated by disc diffusion method against gram-negative bacteria *Escherichia coli*. Amongst the extracts tested, the *Datura stramonium* L. and *Ocimum basilicum* L. showed significant antibacterial activity against the bacterial pathogen. *D. stramonium* showed the highest antibacterial activity followed by *O. basilicum* extract. Phytochemical analysis of extracts showed the presence of alkaloids, tannins, saponins, flavonoids, and phenols. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ABTS analysis of potent extract *D. stramonium* showed appreciable antioxidant activity. For DPPH and ABTS assay the highest antioxidant activity was observed at 60 mg ml^{-1} concentration with a percent inhibition of 71.66 and 77.19 with IC50 value 9.71 and 22.16 µg ml^{-1} respectively. Moreover, the radical scavenging activity of the extract was lower than that observed for the synthetic antioxidants butylated hydroxyanisole (BHA)and butylated hydroxytoluene (BHT). The total phenolic content of *D. stramonium* extract was found to be 0.592 mg GAE/gm which was assayed using Folin ciocalteau reagent. The results provide evidence that the extract of *D. stramonium* and *O. basilicum* can be further recommended in the treatment of the infections caused by E. coli; further *D. stramonium* is also a potential source of natural antioxidants.

Keywords: Antibacterial, Antioxidant, Phenolic content, Plant extracts.

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Introduction

From antiquity, nature has been a rich store of remedies for relief from various ailments affecting mankind. Plants have been used for thousands of years in traditional medicine. The use of plants for treating diseases is as old as the human species. Plants produce a wide variety of secondary metabolites such as vitamins, terpenoids, tannins, flavonoids, alkaloids, and other metabolites, which are rich in antimicrobial and antioxidant activities (Wong et al., 2006; Baker et al., 2010). Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely known.

Many plants have been documented for their biological (Grover *et al.,* 2002; Gajera *et al.,* 2005) and antimicrobial (Arora, 1998; Polambo and Semple, 2001) properties. It can be assumed, that although the bulk of traditional antibiotics can still manage drug-resistant bacteria, many commonly used antibiotics are no longer effective (Levy, 1998; Wright, 2010). Bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Drug resistance can be described as a state of decreased sensitivity to drugs that ordinarily cause growth inhibition or cell death. More strains of pathogens have become antibiotic-resistant, and some have become resistant to several antibiotics and chemotherapeutic agents, the phenomenon of multidrug resistance. Limited treatment options for infections caused by such multiresistant microorganisms prompted the search for novel compounds with a broad spectrum of activity and new therapeutic strategies. To expand the spectrum of antimicrobial agents from natural resources, the plant extract was subjected to assess their antibacterial potential.

Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals". Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and diseases. Consequently, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components (Sies, 1997; Vertuani *et al.,* 2004). Synthetic antioxidants such as BHA and BHT are restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens (Feng *et al.,*2006). Several clinical studies are suggesting that the antioxidants in fruits, vegetables, tea, and red wine are the main actors for the observed efficacy of these foods in reducing the incidence of chronic diseases. Thus, a need for identifying alternative natural and safe sources of food antioxidants has been created, and the search for antioxidants, especially from plant origin, has notably increased in recent years. Several reports have revealed that the majority of the antioxidant activity may be from biochemicals such as flavonoids, isoflavones, flavones, anthocyanins, catechins and other phenolics (Alothman *et al.,* 2009; Isabelle *et al.,* 2010).

Department of Botany, DDU Gorakhpur University, Gorakhpur-273009, INDIA

Corresponding Author: Dr. Pooja Singh, Department of Botany, DDU Gorakhpur University, Gorakhpur-273009, INDIA, Phone: +91-9415083883, Email: pooja.ddu@gmail.com.

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The purpose of the present study is to investigate the antibacterial potential and antioxidant properties of plant extracts.

Material and Methods

Plant materials

The leaves 21 plants were collected from different regions of Gorakhpur district. The leaves were plucked and packed in polythene bags. Plants were initially identified by morphological features and then confirmed from the herbarium database present in the herbarium of DDU Gorakhpur University Gorakhpur. The scientific names and family of the 21 plant materials are detailed in Table 1.

Preparation of plant material

The fresh leaves were washed with tap water and then with 90 per cent alcohol, chopped into smaller pieces with a knife and then kept in the shade for 14 days to dry and then crushed using pestle and mortar and further reduced to powder using electric blender and then stored in airtight closed bottles until tested and analyzed.

Extraction procedure

10 g of the powdered sample of the plant was soaked in 100 ml of methanol in a 250 ml conical flask at room temperature with shaking after every 4 for 24 h. The extract was filtered using a muslin cloth and then Whatman no.1 filter paper. The filtrates were then evaporated to dryness in a rotary evaporator maintained to remove residual solvents and then stored in screw-capped bottles for further use. The extracted powder was resuspended in the methanol at desired concentrations before it was tested for the antibacterial activity.

Microbial strains and preparation of inoculums

Gram-negative bacteria *Escherichia coli* (MTCC No. 3053) was used for evaluation of antibacterial assay. The stock culture was maintained in nutrient agar (NA) slant at 4°C and sub-cultured monthly. Working cultures were prepared by inoculating a loopful of each test microorganism in 10 ml of nutrient broth (NB) from NA slants. Broths were incubated at 37°C for 18-20 hours. The suspension was diluted with sterile distilled water to obtain approximately 10^6 CFU/ml.

Determination of antibacterial activity

The antibacterial activity of plant extracts was evaluated using disc diffusion method (NCCLS, 1997). 10ml of sterilized nutrient agar medium was poured in 80mm Petridishes and was allowed to solidify. The plates were seeded by spreading 0.1 ml of overnight inoculum and allowed to set for 20-25mins. The concentration of the bacterial cultures was maintained to 10^6 colony forming units (CFU)/ml using Mcfarland standard. For, screening, sterile, 6mm diameter filter paper discs were soaked in plant extracts at 100 µg ml^{-1} concentration and placed on the surface of inoculated media agar plates using sterile forceps and then gently pressed down onto the agar surface. Disk soaked with the solvent was used as control. Uninoculated plates were regarded as a negative control. This process ruled out the possibility of contamination. The positive control plates were inoculated with a test organism. All the plates were incubated at 35-37°C for 24 h. Clear inhibition zones around the discs indicated the presence of antibacterial activity. Diameter of inhibition zones were measured in millimeters. An inhibition zone of 10mm or more was considered as high antibacterial activity.

Table 1: Antibacterial activity of different plant extracts against the bacterial strains tested based on Disc Diffusion Method.

Plant extracts	*Family*	*Part used*	*Zone of inhibition*
Anisomelos indica (L.) Kuntze	Lamiaceae	Leaf	14.33±0.46
Antigonon leptopus Hook. and Arn.	Polygonaceae	Leaf	-
Cannabis sativa L.	Cannabaceae	Leaf	10±0.81
Catharanthus roseus (L.) G. Don	Apocynaceae	Leaf	14±0.81
Datura stramonium L.	Solanaceae	Leaf	30±0.45
Erigeron bonariensis L.	Asteraceae	Aerial parts	-
Fleurya aestuans (L.) Gaudich.	Urticaceae	Leaf	-
Legistromea speciosa (.L) Pers.	Lythraceae	Leaf	-
Leonurus sibiricus L.	Lamiaceae	Aerial parts	-
Leucas aspera Willd.	Lamiaceae	Leaf	10±0.161
Lippia alba Mill.	Verbenaceae	Leaf	11.33±0.46
Moringa olifera Lam.	Moringaceae	Leaf	-
Ocimum basilicum L.	Lamiaceae	Aerial parts	16.67±0.94
Ocimum gratissimum L.	Lamiaceae	Aerial parts	-
Ocimum sanctum L.	Lamiaceae	Aeria; parts	-
Oxalis corniculata L.	Oxalidaceae	Aerial parts	-
Phyllanthus niruri L.	Phyllanthaceae	Aerial parts	-
Putranjeeva roxburghii Wall.	Putranjivaceae	Leaf	13.33±0.46
Syzygium cumini (L.) Skeels.	Myrtaceae	Leaf	-
Tamarindus indicus L.	Fabaceae	Leaf	-
Tectona grandis	Lamiaceae	Leaf	-

Determination of MIC (minimum inhibitory concentration) values

The minimum inhibitory concentration value for bacterial pathogen was determined by agar dilution technique of CLSI with slight modifications ((NCCLS, 2002). A series of two folds dilution of extract concentrations (25-3200 µg ml^{-1}) was prepared in Petridishes. 10ml of sterilized and molten nutrient agar medium was poured in each dish already containing 100µl amount of extracts with different concentrations. Plates were dried at 35ºC for 30 min before spot inoculation with 5 µl of overnight bacterial culture (adjusted to 0.5 MacFarland standard) containing approximately 10^5 CFU/spot using a sterilized inoculating loop. Nutrient agar with solvent was used as positive control and similar uninoculated nutrient agar plates were kept as a negative control. The inoculum spots were allowed to dry at room temperature and plates were incubated at 35-37ºC for 24h. MICs were determined as the lowest concentration of oil inhibiting the visible growth of microorganisms on agar plate disregarding the presence of 1 or 2 colonies.

Determination of MBC (minimum bactericidal concentration) values

The MBC of the extracts was determined as described by (Mishra *et al.,* 2008). Fresh nutrient agar medium was poured into petriplates and allowed to solidify. Inoculum from various poisoned plates of MIC experiment showing no growth was submitted to subculture on freshly prepared plates. The lowest concentration of antimicrobial agent from which bacteria do not recover on the fresh medium was treated as MBC.

Phytochemical analysis of extract

To test for the presence of the active ingredients in the test sample the phytochemical analysis of extract was done according to the methods of Harborne (1978) with slight modifications.

Test for alkaloids

Each plant sample (0.5 g) was dissolved 2% HCl in a steam bath and filtered. Three different methods were used. This mixture was allowed to cool and then filtered. The filtrate was shared in equal proportion into three test tubes and labeled A, B, C. Turbidity or precipitation was taken as evidence for the presence of alkaloids. 1 ml of the above filtrate was treated with few drops of Mayer's reagent giving rise to a cream or pale yellow precipitate. Another 1 ml of filtrate was treated with a few drops of Dragendoff's reagent giving rise to a reddish-orange precipitate. Lastly, 1 ml of filtrate was treated with Wagner's reagent giving rise to a brown or reddish-brown precipitate.

Test for tannins

About 0.5 g of the dried powdered samples was boiled in 20 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of an emulsion.

Test for flavonoids

- *NaOH test:* A small amount of extract was treated with aqueous NaOH and HCl, observed for the formation of yellow-orange color.
- *H_2SO_4 test:* A fraction of extract was treated with concentrated H_2SO_4 and observed for the formation of orange colour.
- *Lead acetate test:* A small amount of extract was treated with lead acetate and observed for the formation of white precipitate.

Test for steroids

Two milliliters of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2S0_4. The color changed from violet to blue or green in the samples indicating the presence of steroids.

Test for terpenoids

Extract (1ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark green color.

Test for phenols

Ferric chloride test: The fraction of extract was treated with 5 % ferric chloride and observed for the formation of deep blue or black color.

Test for carbohydrates

Molisch's test: Few drops of Molisch's reagent was added to each of the portion dissolved in distilled water, this was then followed by addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet color at the interphase of the two layers was a positive test.

Test for Anthraquinones

Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia; pink or deep red colorations of aqueous layer indicate the presence of anthraquinone.

Determination of antioxidant activity

a. DPPH free radical scavenging activity

Effect of extracts on DPPH radical was estimated using the method of Güllüce *et al.* (2003) with slight modifications. 0.004% of DPPH (Hi Media) was prepared in methanol and 2ml of this solution was mixed with different concentrations of extracts (10, 20, 30, 40, 50 and 60 µg ml^{-1}) dissolved in methanol. The reaction mixture was vortexed thoroughly and left for 30 mins. After 30mins absorbance of the mixture was measured at 517 nm in a UV spectrophotometer (Hitachi) against a blank (pure methanol). A control sample was also prepared as above without any oil. Ascorbic acid, BHT and BHA was taken as reference standards. Experiments were performed in triplicate and averaged. The IC_{50} value was determined from percent inhibition versus concentration graph. Percent inhibition was calculated from control using the following equation:

$$\text{Radical scavenging activity (\%)} = \frac{[\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}]}{\text{Abs}_{\text{control}}} \times 100$$

Where:

$Abs_{control}$= Absorbance of DPPH radical + methanol.

Abs_{sample} = Absorbance of DPPH radical + sample extract/standard

b. ABTS radical scavenging assay:

For ABTS assay, the method of Adedapo *et al.* (2008) was adopted. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. 1ml of plant extract (10, 20, 30, 40, 50 and 60 µg ml^{-1}) were allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of BHT and BHA and percentage inhibition calculated as ABTS radical scavenging activity.

$$\% \text{ inhibition} = \frac{[Abs_{control} - Abs_{sample}]}{Abs_{control}} \times 100$$

Where:

$Abs_{control}$= Absorbance of ABTS radical + methanol

Abs_{sample} = Absorbance of ABTS radical + sample extract/standard

c. Determination of total phenolic contents

The total phenolic content of *D. stramonium* extract was determined according to the method of Muruhan *et al.* (2013) with slight modifications using Gallic acid as the standard. 1 ml of various concentrations of *D. stramonium* (100, 200, 300, and 400 µg ml^{-1}) were mixed with 2 ml of 7.5% Na_2CO_3 and after 2 min 0.1 ml of Folin Ciocalteau (Hi Media) reagent (diluted tenfold with distilled water) was added and mixed well. After 30mins incubation, the absorbance of mixtures was recorded spectrophotometrically at 750nm. The total phenolic contents were calculated as gallic acid equivalent (GAE) from a calibration curve of gallic acid standard solutions and expressed as mg of gallic acid per gm of extract sample.

Results

Antibacterial activity

Results from antibacterial disc diffusion assay are summarized in Table 1. Some of the extracts showed moderate to high inhibiting activity while most of the extracts did not found effective against tested the tested bacterial pathogen. The zones of inhibition ranged from 10-30mm. Results showed that *Datura stramonium* and *Ocimum basilicum* extracts showed significant antibacterial activity against the bacteria tested. Additionally, the extracts of *Anisomeles indica* and *Catharanthus roseus* also showed moderated inhibitory activity. *D. stramonium* showed the highest activity forming 30mm zone of inhibition against *Escherichia coli* followed by *O. basilicum* which formed 16.67mm inhibition zone. The zone of inhibition formed by other extracts was negligible. Furthermore, the antibacterial activity of the most effective extract against *E. coli* quantitatively was assayed by determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

D. stramonium exhibited strong action against *E. coli* with MIC value of 800 µg ml^{-1} followed by the extract *O. basilicum* with 1600 µg ml^{-1} MIC value (Table 2). MBC values were found to be 1600 and 3200 µg ml^{-1} for *D. stramonium* and *Ocimum basilicum* respectively (Table 2).

Phytochemical analysis

The preliminary phytochemical screening with the various qualitative chemical tests revealed the presence of various secondary metabolites like Alkaloids, tannins, saponins, flavonoids, tannins. The results were shown in Table 3.

Antioxidant activity

During an assessment of the antibacterial potential of an extract; *Datura stramonium* was most potent, thus was subjected to further investigation of its antioxidant properties.

DPPH radical scavenging assay

The DPPH radical scavenging activity of the most potent extract, *D. stramonium* and references are shown in Figure 1. *D. stramonium* methanolic extract notably reduced the concentration of DPPH free radical, with an efficacy lower than that of reference BHA (Butylated hydroxyl anisole) and BHT (Butylated hydroxytoluene). The results showed a significant decrease in the concentration of DPPH free radical due to the scavenging ability of extract and reference. The decrease in the concentration of DPPH was observed with the increase in the concentration of extract. The highest antioxidant activity was observed at 60 mg ml^{-1} concentration (75.66%). The IC50 value of extract was found to be 9.71 µg ml^{-1}.

ABTS radical cation decolorization assay

The results are demonstrated in Figure 2. The results showed that

Table 2: MIC and MBC data of *D. stramonium* and *O.basilicum* extracts against *E. coli* in µg ml^{-1}.

Bacterial strain	*D. stramonium*		*O. basilicum*	
	MIC	MBC	MIC	MBC
E. coli	800	≤1600	1600	≤3200

Table 3: Results of phytochemical screening.

Test	*D. stramonium*	*O. basilicum*
Alkaloids		
Dragendroff's Test	+	+
Mayers's Test	+	+
Hagers's Test	+	+
Tannins	+	+
Saponins	+	+
Flavonoids		
NaOH Test	+	+
H_2SO_4 Test	+	+
Lead acetate Test	+	+
Steroids	-	-
Terpenoids	-	-
Phenols	+	+
Carbohydrates	-	-
Anthraquinones	-	-

Table 4: Total phenolic content and antioxidant activity regarding to IC_{50} value of plant extract.

Plant extract	*Phenolic content (mg GAE/gm)*	*IC_{50} value in µg ml^{-1} (DPPH assay)*	*IC_{50} value in µg ml^{-1} (ABTS assay)*
D. stramonium	0.592	9.71	22.16

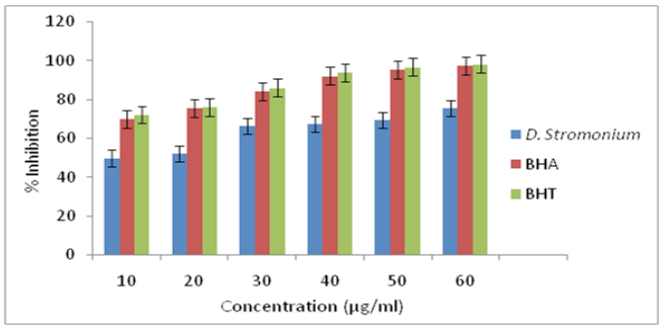

Fig. 1: Free radical scavenging activity of *D. stramonium* extract and reference antioxidants at different concentrations showing percent inhibition of DPPH radical.

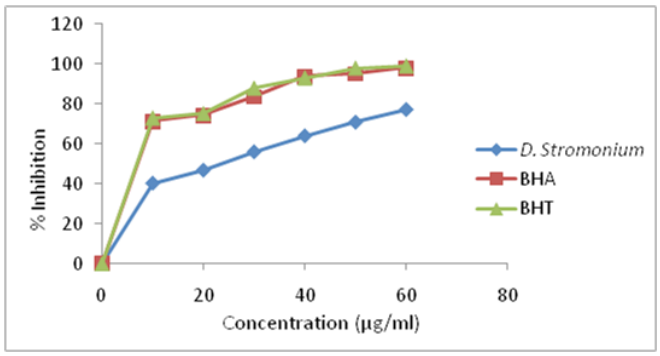

Fig. 2: Total antioxidant activity of *D. stramonium* extract and reference antioxidants by ABTS radical cation decolorization assay.

Datura extract was found to be a good scavenger of ABTS free radicals. The rise in the concentration of the extract was found to enhance the radical scavenging ability. At 60 µg ml^{-1} concentration, the highest activity of extract was observed. Percent inhibition was 77.19%, 95.66% and 94.82% for *Datura* extract, BHA and BHT respectively. In ABTS scavenging assay the IC_{50} value of *D. stramonium* extract was found to be 22.16 µg ml^{-1}.

Total phenolic content

Phenolics are the class of compounds, which acts as free radical scavengers and are responsible for the antioxidant activity in many plants. The total phenolic contents in *D. stramonium* was measured by Folin- Ciocalteau reagent expressed as Gallic acid equivalent (GAE) in mg g^{-1} (standard curve equation y=0.0059x+0.0579, R^2= 0.9255). The total phenolic equivalent ranged from 0.256 to 0.592 mg GAE/gm at different concentrations (Table 4). The highest total phenolic content was observed at 500 µg ml^{-1} concentration (0.592 mg GAE/gm).

Discussion

Antibacterial activity

Out of 22 extracts tested, only methanolic extracts of *D. stramonium* and *O. basilicum* exhibited good antibacterial activity and gave a zone of inhibition against *E. coli*. Eloff (1998) reported that methanol was the most effective solvent for plant extraction than any other solvents. Soniya *et al.* (2013) also found methanol as the most effective solvent. *D. stramonium* showed the highest inhibitory activity against a bacterial pathogen. The present study is comparable with the reports of Sharma and Sharma (2013) and Gachande and Khillare (2013) and Sreenivasa *et al*. (2012). *O. basilicum* extract was proved good in inhibiting *E. coli* after *D. stramonium* as reported by Hossain *et al*. (2010). It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability, unavailability of vital intracellular constituents and impairment of bacterial enzymes. Though the minimum inhibitory concentration is high, nevertheless it showed that plant extract under in-vitro study has broad antibacterial activity. Generally, it is well known that gram-negative bacteria are more resistant than gram-positive bacteria. However, in this study *E. coli*, a gram-negative bacteria was found sensitive to the extract of *D. stramonium* followed by *O. basilicum*.

Phytochemical analysis

The constituents of the extracts of *D. stramonium* (dried) leaves contain compounds which may be responsible for their observed antioxidant and antibacterial activities. These are phenolic compounds, alkaloids, flavonoids, tannins, and saponins. Alkaloids have established broad-spectrum antibacterial activity and are also used as analgesics and narcotics for pain relief. This supports earlier findings which reported the anti-inflammatory action of *Fagara*, a known antisickling phytomedicine (Elekwa *et al*., 2008). Flavonoids and phenolics found in the extracts are suggestive of their antioxidant property. Phenolic compounds make up one of the major families of secondary metabolites in plants. Flavonoids enhance the effects of vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses, and other microbes, allergies, and inflammation. Tannins are found in almost every plant part: bark, wood, leaves, fruits, and roots (Scalbert, 1991). Many human physiological activities, such as stimulation of phagocytic cell, host-mediated tumor activity and a wide range of anti-infective actions, have been assigned to tannins (Haslam, 1996). Tannins can be toxic to filamentous fungi, yeasts, and bacteria (Scalbert, 1991). Saponins are glycosides with foaming characteristics. They are common in a large number of plants and plant products that are important in human and animal nutrition (Francis *et al*., 2002).

Total phenolic content and antioxidant activity

Oxygen is one of the most essential components for living, it is also a double-edged sword. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals''. Cell damage caused by free radicals appears to be a major contributor to aging and diseases like cancer, heart disease, a decline in brain function, a decline in the immune system, etc. Due to the negative and toxic effects of synthetic antioxidants, natural phenolic antioxidants are being promoted as food preservatives and diet supplements (Botsoglou *et al*., 2002; Shetty, 1997).

DPPH is a stable free radical which accepts an electron or hydrogen radical to become a stable diamagnetic molecule, which is widely used to investigate the radical-scavenging activity. In

DPPH radical scavenging assay, antioxidants react with DPPH, and convert it to yellow colored *α,α*-diphenyl-*β*-picryl hydrazine. The degree of discoloration indicates the radical-scavenging potential of antioxidant activities (Blois, 1958). Figure 1 shows DPPH scavenging activity of *D. stramonium* extracts at different concentrations in comparison with well known synthetic antioxidants. The antioxidant activity reflected by the DPPH radical scavenging assay was observed in the methanolic leaf extract of *D. stramonium*.

The ABTS assay is based on the inhibition by antioxidants of the absorbance of radical cation ABTS, which has a characteristic long-wavelength absorption spectrum. Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical has characteristic absorbance maximum at 734 nm which decreases with the scavenging of proton radicals (Mathew and Abraham, 2006). Generation of ABTS radical cations forms the basis of one of the spectrophotometric methods that have been applied to the measurement of total antioxidant activity of various substances. Higher concentrations of the extract were more effective in quenching free radicals (Fig. 2).

In DPPH as well as ABTS assay, the inhibition was found to be concentration-dependent. Percent inhibition was found directly proportional to the concentrations of extract i.e. with the increase in concentration there is also an increase in percent inhibition. The presence of phytochemicals such as phenolic compounds, flavonoids may directly contribute to the antioxidant activity of the extract.

Many studies demonstrated a correlation between phenolic content and antioxidant activity (Yang *et al.,* 2002). On the other hand, Bajpai *et al*. (2005) reported no correlation between total phenolic content and antioxidant capacities of many medicinal plant extracts. The phenolic compounds may contribute directly to the antioxidative action and are also good hydrogen donors, which makes them good antioxidants (Dudonne *et al.,* 2009). Total phenolic content was found to be 0.592 mg GAE/gm. To a certain level, we can establish a correlation between total phenolic content and antioxidant activity in the present study conducted.

D. stramonium extract was found to have promising antioxidant activity as a well good antibacterial activity along with an appreciable amount of total phenolic content. The DPPH and ABTS both the assays proved that the antioxidant activity of the extract was appreciable. It can be used as a potential source of natural antioxidants.

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