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Evaluation of antibacterial and antioxidant activities of *Abutilon indicum* and *Datura stramonium*

V. Soundaryadevi and V. Jeyamanikandan*

Department of Biotechnology, Jeppiaar Engineering College, Chennai, Tamilnadu, India

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ABSTRACT

Medicinal plants have a promising future because there are about half million plants around the world, and their medical values still remains unexplored much, and their medical activities could be decisive in the treatment of present or future studies. The current study is undertaken to ascertain the antibacterial activity of crude with step gradient solvent of methanol, acetone, petroleum ether in leaves of Abutilon indicum and Datura stramonium. The extracts were prepared in methanol (M) and petroleum ether (P) and in Methanol (M) and acetone (A) in various proportions M:P and M:A viz. 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100%. These extracts were examined for antibacterial activity using agar well diffusion method against four bacterial pathogens, Bacillus cereus, Staphylococcus aureus, Escherichia coli and Salmonella typhi. Among the tested pathogens B. cereus, S. typhi and S. aureus were found to be the most susceptible to the combined extract. The M:P (80:20) and M:A (0:100) of combined extract shown highest activity in S. typhi, B. cereus and S. aureus. These proportion were subjected to free radical scavenging activity on DPPH and the result showed that 100% acetone extract have showed highest scavenging potential. The results reveals from this study would support the use of natural plant as a cheap, environmentally safe and reliable source for the treatment of diseases.

Key words: Antibacterial activity, *Abutilon indicum*, *Datura stramonium*, Antioxidant activity.

INTRODUCTION

In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-drug resistant (MDR) [6, 7, 16]. Even though pharmaceutical companies have produced a number of new antibacterials in the last years, resistance to these drugs has increased and has now become a global concern [4]. Due to the increase of resistance to antibiotics, there is an urgent need to develop new and innovative antimicrobial agents. Plants contain many bioactive compounds that can be of interest in therapeutic.

Medicinal plants are gifts of nature to cure limitless number of diseases among human beings [2]. The abundance of plants on the earth's surface had led to an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents [5]. The use of herbal medicines is steadily growing with approximately 40% of population reporting use of herbs to treat medical illness within the past year [15].

Abutilon indicum genus of the *Malvaceae* family comprises about 150 annual or perennial herbs, shrubs or even small trees widely distributed in the tropical and subtropical countries of America, Africa, Asia and Australia. Some

of the plants belonging to the species are amongst much acclaimed Ayurvedic herbs and in the recent past there has been a renewed scientific interest in exploring the species [11].

Datura stramonium (*D. stramonium*) is one of the widely well-known folklore medicinal herbs. The troublesome weed, *D. stramonium* is a plant with both poisonous and medicinal properties and has been proven to have great pharmacological potential with a great utility and usage in folklore medicine. *D. stramonium* has been scientifically proven to contain alkaloids, tannins, carbohydrates and proteins. This plant has contributed various pharmacological actions in the scientific field of Indian systems of medicines like analgesic and antiasthmatic activities [10].

Medicinal plants possess immune-modulatory and antioxidant properties, leading to antimicrobial activity. Plant derived natural products such as flavonoids, terpenes, alkaloids has been received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemo-protective effects [14]. Several plant products have been tested for antibacterial and antitumor activity. Over 50% of the drugs in the clinical trial for antitumor activity were isolated from the natural source or are related to them.

Antioxidants are phyto-chemicals, vitamins and other nutrient that protect our cells from damage caused by free radicals. An Antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent [1].

The traditional users own only ideas on the identification of the plant and dosages through personal practice but are not with awareness on scientific reason behind its medicinal uses [3]. Thus in the search for cheap, low toxic and more effective antimicrobial potential among materials of plant origin, the present study was designed to evaluate the *in vitro* antimicrobial and antioxidant activity of the medicinal plants.

EXPERIMENTAL SECTION

Sample collection

The fresh leaves of *A.indicum* and *D.stramonium* were collected from Kanchipuram district, Tamilnadu.

Preparation of plant extracts

The leaves of *D. stramonium* and *A. indicum* were washed with tap water and then with distilled water. The samples were kept in the room temperature for two weeks. The dried samples were grounded into a fine powder using grinder mixer. Each 25 grams of *D. stramonium* and *A. indicum* fine powder was soaked in Methanol (M) and Petroleum ether (P) and in Methanol (M) and Acetone (A) separately in various proportions M:P and M:A viz.100:0 80:20,60:40, 40:60, 20:80 and 0:100%. It was kept in the orbital shaker for 24 hours at room temperature. These extract were filtered through Whatmann No. 1 filter paper [13]. The filtrate was allowed to dryness and resuspended in Dimethyl sulfoxide (DMSO).

Experimental Microorganism

Four different bacterial strains used for the study were *Salmonella typhi*, *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli*. The strains were obtained from Basic Biomedical Science, Bharathidasan University, Trichy. The stock culture was maintained on nutrient agar slant and incubated at 4°C.

Determination of Antibacterial Activity

Antibacterial activity was assayed by the well diffusion method [9]. Pathogenic bacterial strains were inoculated in sterile broth and incubated at 37°C for 24 hours. Pathogens were swapped on the surface of sterile petridishes in 20 ml of solidified nutrient agar. The control and the experimental samples were placed in the sterile solidified nutrient agar petriplates to assess the effect of solvent and extracts on pathogens [8] .These agar plates were incubated at 37°C for 24 hours and the antibacterial activity was measured accordingly based on the inhibition zone around the well impregnated with plant extract. Antibacterial activity was expressed in diameter zone of inhibition, which was with the outer side of the well to inner side of the inhibition zone.

Antioxidant Activity Assay

Estimation of Radical Scavenging Activity (RSA) using the DPPH assay:

The Free Radical Scavenging Activity of different extracts was determined by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay according to Nenadi's (2002) [12] with small modification. The decrease of the absorption at

517nm of the DPPH solution after the addition of the antioxidant was measured in a cuvette containing 2960 µl of 0.1 mM ethanolic DPPH solutions mixed with 40 µl of 20 to 100µg/ml of plant extract and vortexed thoroughly. The setup was left at dark in room temperature and the absorption was monitored after 20 minutes. The ability of the sample extract to scavenge DPPH radical was calculated by the following equation:

$$\% \text{ of DPPH Radical Scavenging Activity (\% RSA)} = \frac{\text{Abs. control} - \text{Abs. sample}}{\text{Abs. control}} \times 100$$

Abs. control is the absorbance of DPPH radical + ethanol; Abs. sample is the absorbance of DPPH radical + sample extract.

RESULTS AND DISCUSSION

Antibacterial Activity

Antibacterial activity of *A.indicum* and *D. stramonium* was determined against four bacterial pathogens using agar well diffusion method. The results revealed that the crude methanol extract of *A.indicum* showed the highest activity (20mm) against *S.typhi* and the least (16mm) against *B. cereus* (16mm) and for *D. stramonium* crude methanol extract shown the highest activity (25mm) against *B. cereus* and the least (12mm) against *E.coli*. Among the step gradient solvents extracts, of the four bacterial pathogens tested, maximum numbers of pathogens were inhibited in 80:20% Methanol:Petroleum ether extract of both the plants. The highest activity was presented against *B. cereus* (26mm) and also in *S.aureus* (22mm). In the same way, Methanol:Acetone (0:100%) extract shown highest activity against three bacterial strains *B. cereus* (24mm), *S. typhi* (18mm) and *S. aureus* (24mm) Whereas, no activity was found against *E.coli* in both the plant samples. The antibacterial activity of the extracts was shown in the table 1 and their graphical representations were shown in the figure 1-4.

Table 1: Antibacterial activity of crude extract and the step gradient extract activity of *A.indicum* and *D. stramonium*

Plants	Solvents used for Extracts	Zone of Inhibition (mm)			
		E.coli	***S.aureus***	***B.cereus***	***S.typhi***
Negative control	Solvents	-	-	-	-
Abutilon indicum	Methanol	2	5	16	20
	Acetone	-	7	10	3
	Petroleum ether	-	2	8	5
A.indicum* + *D.stramonium	**M:P (%)**				
	100:0	-	12	18	14
	80:20	-		26	22
	60:40	-	10	15	10
	40:60	-	8	10	-
	20:80	-	-	7	-
	0:100	-	-	-	-
Positive control	Gentamycin	22	22	25	24
D. stramonium	Methanol	12	8	25	10
	Acetone	-	10	7	5
	Petroleum ether	-	-	-	-
A. indicum* + *D. stramonium	**M:A (%)**				
	100:0	-	20	22	24
	80:20	-	18	18	16
	60:40	-	14	10	13
	40:60	-	8	9	8
	20:80	-	9	7	6
	0:100	-	24	24	18

(--) – indicates no antimicrobial activity against the respective bacteria

Figure 1: Antibacterial activity of *A.indicum* against different organisms

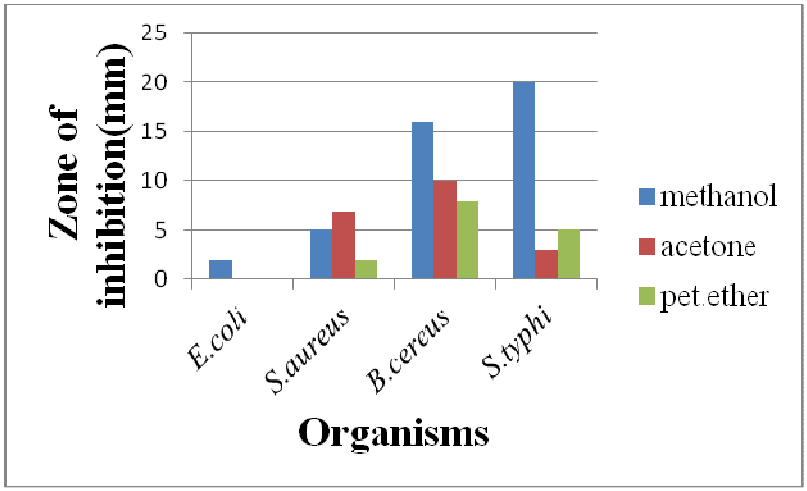


Figure 2: Antibacterial activity of *D.stramonium* against different organisms

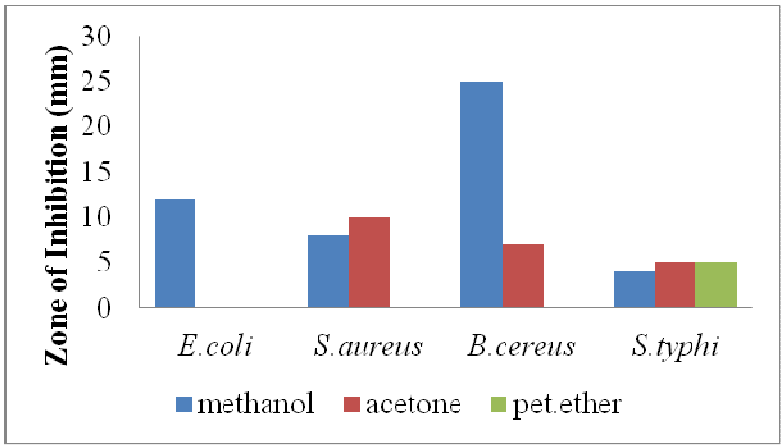


Figure 3: Antibacterial activity of *A.indicum* + *D.stramonium* (M:P) against different organisms

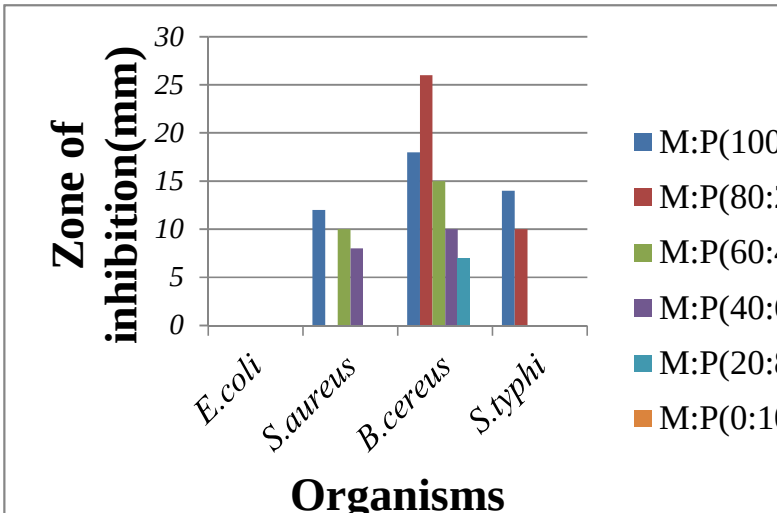
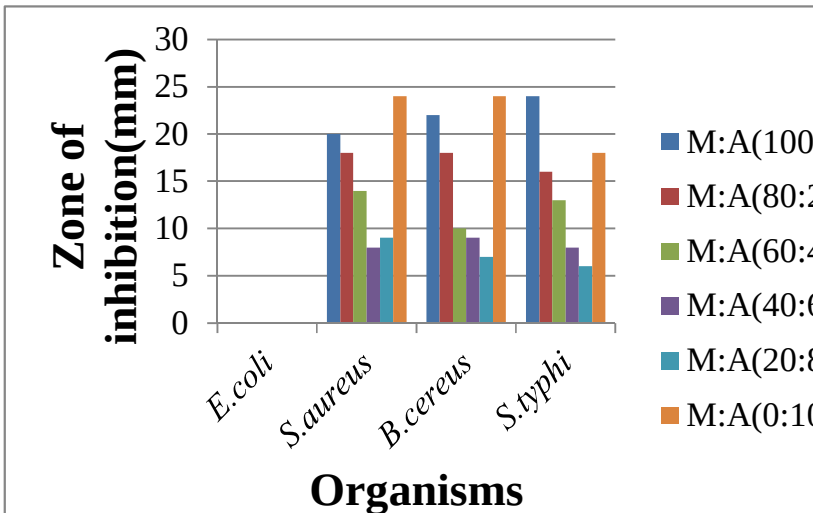


Figure 4: Antibacterial activity of *A.indicum* + *D.stramonium* (M:A) against different organisms



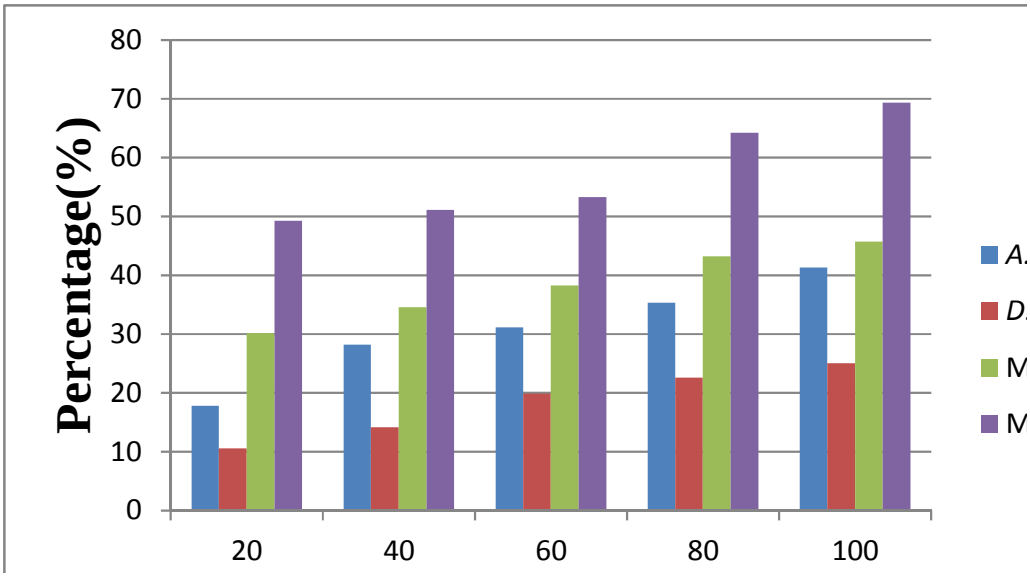
Antioxidant Assay

The plant extracts which showed maximum activity against bacterial pathogens were taken to assess its free radical scavenging potential using DPPH method. The plant extract *A.indicum* + *D.stramonium* for methanol: acetone (0:100) showed highest activity 69.33% at 100µg/ml and lowest activity was recorded for *D.stramonium* at 20 µg/ml. All plants showed inhibition against DPPH even at 20 µg/ml. The results of the percent scavenging on DPPH by different plants extracts are given in Table 2 and graphically represented in figure 5.

Table 2: Free radical scavenging (DPPH) activity of *A.indicum* and *D.stramonium*

Plant extracts (µg/ml)	*A.indicum* (%)	*D.stramonium* (%)	M:P (80:20) combined extract (%)	M:A (0:100) combined extract (%)
20	17.8	10.54	30.18	49.25
40	28.2	14.11	34.57	51.10
60	31.1	19.85	38.25	53.29
80	35.3	22.6	43.22	64.20
100	41.28	25.04	45.7	69.33

Figure 5: Free radical scavenging (DPPH) activity of *A.indicum* and *D.stramonium*



CONCLUSION

The present study demonstrated that methanol, methanol: petroleum ether (80:20) and methanol: acetone (0:100) extract found to be the effective for antibacterial and antioxidant activity. The crude methanol extracts of *A.indicum* and *D.stramonium* have not showed maximum activities when compared to that of step gradient solvent extracts. The results of the present study support the traditional use of the studied plants in the treatment of bacterial infections. It also provides useful baseline information for the potential use of the studied plants in the fight against both sensitive and MDR phenotypes.

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