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Research Paper

ANTIBACTERIAL ACTIVITY OF LEAF AND SEED EXTRACTS OF *DELONIX REGIA* AND *ACHYRANTHUS ASPERA* AGAINST SELECTED BACTERIAL STRAINS

Dhanalakshmi D[1*] and Manimegalai K[1]

*Corresponding Author: **Dhanalakshmi D,** ✉ dhanavenkatshree@gmail.com

The antibacterial activity of petroleum ether, chloroform and ethanol extracts of the leaf and seed of *Delonix regia and Achyranthus aspera* were evaluated using agar well diffusion method against five bacterial strains namely, *Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Micrococcus luteus, Pseudomonas aeruginosa.* Chloramphenicol was used as standard. Among the three solvents analysed in leaf and seed, chloroform seed extract of *D. regia* and ethanol seed extract of *A. aspera* exhibited a high inhibitory zone against *E. coli* than other bacterial strains. Hence the phytoconstituents present may be used as antibiotic drugs.

Keywords: Delonix regia, Achyranthus aspera, antibacterial activity, chloramphenicol

INTRODUCTION

Natural products perform various functions and many of them have interesting and useful biological activities (Harvey, 1999). Plant species being used in various human cultures around the world for medicinal purpose. Use of herbal medicine represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases (Edeoga *et al.,* 2005).

Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular for 80% of world population (Bibitha *et al.,* 2002; Maghrani *et al.,* 2005). Recognition and development of the medicinal and economic benefits of traditional medicinal plants is on the increase in both developing and industrialized countries (Zhang, 1998).

Plants and plant products have been used extensively throughout history to treat medical problems. Numerous studies have been carried out to extract various natural products for screening antimicrobial activity, but attention has not been focused intensively on screening of bioactive compounds of these products for their antimicrobial activity (Nita, 2002; Velickovic

[1] Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore - 43.

et al., 2003; Cowan, 1999; Sakagami, 2001; Ateb, 2003).

The therapeutic properties of various medicinal plants have been used to treat human diseases. It has been estimated that between 60 – 90% of the population of developing countries use traditional and botanical medicines (WHO, 2002). Medicinal plants contain substances that can be used for therapeutic purposes or which are used as precursors for the synthesis of useful drugs (Soforowa, 1993). They are of great importance to the health of the individuals and communities and they play a significant role in providing primary health care services to rural people. They also serve as therapeutic agents as well as important raw materials for the production of traditional and modern drugs.

Increasing the failure of synthetic drugs, side effects and development of antibiotic resistance by pathogenic microorganisms leads to development of the identification and screening of several medicinal plants for their potential antimicrobial activity (Iwu *et al.,* 1999). Thousands of species are known to have medicinal value and several studies reported the antimicrobial activity of plants (Werner *et al.,* 1999 and Samy and Ignacimuthu, 2000). The value of traditional medicine has recognized by World Health Organization and involved in creating strategies, guidelines and standards for plant medicines (WHO, 2002). In pharmaceutical industries, due to varied medicinal properties in all plant parts include root, stem, flower, fruit is used for extract as drugs.

MATERIALS AND METHODS

Collection of Test Materials

Leaves and seeds of *D. regia* were collected from Tamil Nadu district, India located at 11^0N Latitude and 77^0E longitude.

Preparation of Leaf and Seed Powder

Fresh leaves were collected, washed in water and air dried under shade. Dried leaves were powdered using an electric pulverizer. Fine powder was obtained by sieving. Ripe fruits that had fallen on the ground were collected. The seeds were separated, washed in water and dried under shade. After drying for four weeks, the seeds were ground in an electric pulverizer to get the powder.

Preparation of Extracts

10 g of each of the leaf powder or the seed powder was weighed using an electronic balance (Denver XS-210) and made into packets using Zerohaze filter paper (A Grade, SD's). These powders were subjected to extraction (Harbourne, 1973 and Vogel, 1978). Petroleum ether (60 – 80^0C) extraction was followed by chloroform extraction and ethanol extraction so that the powders were subjected to extraction with solvents of increasing polarity.

Test Microorganism

The five bacterial strains used in the present study were the clinical isolates obtained from P.S.G. Hospitals, Coimbatore. The bacterial strains used were *Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Micrococcus luteus and Pseudomonas aeroginosa.* The effect of various plant extracts on the several bacterial strains was assayed by agar well diffusion method.

Procedure

Petriplates containing 20 ml Muller Hinton medium were seeded with 24 h culture of bacterial strains. Well were cut and 20 ml of the plant extracts (namely petroleum ether, chloroform and ethanol

extracts) were added. The plates were then incubated at 37^{0}C for 24 h. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around well (NCCLS, 1993). Chloramphenicol was used as positive control.

RESULTS AND DISCUSSION

The antibacterial activity of leaf and seed extracts of *D. regia and A. aspera* were screened *in vitro* by agar well diffusion method using chloramphenicol as the standard positive control against selected bacterial strains.

The results of leaf and seed extracts of *D. regia and A. aspera* using extracts of petroleum ether, chloroform and ethanol were shown in Tables 1 and 2 respectively.

Among the three solvents used in the leaf extract of *D. regia* petroleum ether leaf extract against *E. coli* (24 ± 1) possess maximum zone and minimum inhibition was observed in ethanol extract against *E. aerogenes* (8.33 ± 1.52). Similar observations were made by Sridhar *et al.* (2011) in which the ethanolic extract of leaf showed highest activity than seed and root.

In seed extract maximum inhibitory was found in the chloroform extract against *E. coli* (35 ± 1) and the less activity was shown in petroleum ether against *B. subtilis* (10 ± 1). Similar observations were recorded by Omonkhelin *et al.* (2007) which showed that ethanolic extracts of *Kigelia africana* has minimum inhibitory concentration of 6.25 ± 1.07 mg/ml and 7.92 ± 1.52 mg / ml for *S. aureus* and *C. albicans.*

Of the three solvents tested in leaf extract of *A. aspera,* the chloroform leaf extract against *B. subtilis* was observed to have highest zone of inhibition (32.33 ± 1.52) and minimum was observed in ethanol leaf extract against the *B. subtilis and E. coli* (9 ± 1). Similar observations were recorded by Saad *et al.* (2012) in which the antimicrobial activities of the ethyl acetate and methanol extracts showed inhibition activity against both gram positive strains (*S. aureus* (12.55mm) and *B. cereus* (12.5mm)) as well as gram negative bacteria *E. coli* (17.55mm).

Where as in the seed extract of *A. aspera* ethanol extract against *E. coli* possess maximum inhibitory zone (32.33±2.51mm) and minimum

Table 1: Antibacterial Activity of *D. regia* Leaf and Seed Extracts Against Test Organisms

SI. No		Micro Organisms (Diameter of the zone of inhibition in mm)							
		Delonix Regia Leaf Extracts				Delonix Regia Seed Extracts			
		Cont.	PE	CH	E	Cont.	PE	CH	E
1	B. Subitilis	20.66 ± 1.15	21.33 ± 52	21.66 ± 52	19 ± 1	22.33 ± 0.57	10 ± 1	32 ± 1	21.33±1.52
2	E. Coli	22.66 ± 1.15	24 ± 1	17 ± 1	18 ± 2	18.66 ± 0.58	8.33 ± 1.52	35 ± 1	9.66±1.52
3	E. aerogenes	33 ± 1.73	11.66 ± 10.58	9 ± 1	8.33 ± 1.52	25.33 ± 0.58	11 ± 1	29 ± 1	19 ± 1
4	M. luteus	32.33 ± 0.58	19 ± 1	19 ± 1	18.66 ± 1.15	26.66 1.15	18 ± 1	31.66 ± 1.52	10 ± 2
5	P. aeroginosa	29.66 ± 0.58	9.33 ± 1.52	13 ± 1	16 ± 1	42.33 ± 0.57	10.33 ± 2.08	34 ± 1	20 ± 2

Note: Values are mean inhibition zone (mm) ± S.D., Cont. – Chloramphenicol., PE – Petroleum ether. CH – Chloroform, E – Ethanol

Table 2: Antibacterial activity of *Achyranthus aspera* Leaf and Seed Extracts Against The Test Organisms

S. No		Micro Organisms (Diameter of the zone of inhibition in mm)							
		Achyranthus aspera Leaf Extracts				*Achyranthus aspera* Seed Extracts			
		Cont.	PE	CH	E	Cont.	PE	CH	E
1	*B. Subitilis*	22.66 ± 1.15	16.33 ± 1.52	32.33 ± 1.52	9 ± 1	22.33 ± 0.57	20.33 ± 1.52	10 ± 2	31 ± 1
2	*E. Coli*	22.66 ± 1.15	24.33 ± 1.15	31.66 ± 1.52	9 ± 1	18.66 ± 0.58	16.33 ± 0.57	17 ± 1	32.33 ± 2.51
3	*E. aerogenes*	33 ± 1.73	21 ± 1	21.33 ± 1.52	8.33 ± 1.52	25.33 ± 0.58	23 ± 1	16 ± 1	25.33 ± 1.15
4	*M. luteus*	32.33 ± 0.58	20.66 ± 0.58	20 ± 1	9.33 ± 1.52	26.66 ± 1.15	11.66 ± 1.52	13.66 ± 1.52	25.33 ± 1.15
5	*P.aeroginosa*	29.66 ± 0.58	10 ± 1	23.38 ± 1.52	29.66 ± 0.58	42.33 ± 0.57	16.33± 1.52	22± 2	29± 1

was recorded at Chloroform extract against *B. subtilis* (10±2mm).

CONCLUSION

The result of the antibacterial assay showed promising evidence for the antibacterial effect of leaves and seeds of *D. regia and A. aspera.* The plant extracts have great potential as antibacterial compounds against enteric pathogens and they can be uesd in the treatment of enteric infectious. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

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