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Research Article

AN EVALUATION AND COMPARATIVE STUDY OF ROOT EXTRACT OF *ARISTOLOCHIA INDICA* AND ANTIBIOTICS ON *ESCHERICHIA COLI*

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ABSTRACT

Aristolochia indica traditional folklore medicinal plant was screened for antibacterial activity against *Escherichia coli*, the predominant enteric organism causing extra-intestinal infections in man, particularly urinary tract, peritoneum and blood. Sun-dried roots (10g) were homogenized in five different solvents - Acetone, Butanol, DMF, Distilled water and Ether. All five root extracts, solvents and standard antibiotics (Ciprofloxacin 30µg, Nitrofurantoin 300µg, Ofloxacin 5µg, Pefloxacin 5µg and Sparfloxacin 5µg) were tested for antibacterial activity against *E. coli* by disc diffusion method. Solidified Nutrient agar was seeded with 0.5ml inoculum by spread plate technique. The discs impregnated separately with root extracts, pure solvents were introduced on seeded agar plate, similarly the standard antibiotics. The plates were incubated overnight at 37ºC. Growth was determined by measuring the diameter of zone of inhibition expressed in centimeter. The data were subjected statistically to Post Hoc Scheffe test. On comparison of antibacterial assays of pure solvents against root extracts all root extracts exhibited different degrees of activity compared to pure solvents. Ether extract showed maximum activity and butanol extract was least. Acetone, DMF and aqueous extracts activities were same. Among solvents, Butanol and water showed no activity. On comparison of standard antibiotics against root extracts Ciprofloxacin and Ofloxacin activities were higher. Ether extract activity was closer to Nitrofurantoin, followed with Pefloxacin and Sparfloxacin. The study was a preliminary assessment revealing *A. indica* root extracts showed profoundly distinct antibacterial activity and can promise in fighting against enteric infections which are leading cause for Diarrhoea illness and childhood mortality in developing countries.

Keywords: *Aristolochia indica, Escherichia coli,* Antibacterial activity, Standard antibiotics, Enteric infections.

INTRODUCTION

Enteric or diarrhoea infections are major public health problems in developing countries and contribute to the death of 3.3-6.0 million children annually. Among enteric bacteria's *Escherichia coli* is one of the major etiological agents of sporadic and epidemic diarrhoea both in children and adults[1,2]. Also 80% of uncomplicated urinary tract infections are caused by *Escherichia coli*, followed by *Staphylococcus saprophyticus* in as many as 5% to 15% of cases[3,4]. Fluoroquinolones (Ciprofloxacin, Ofloxacin, Sparfloxacin), Nitrofurantoin antimicrobial agents are most commonly used to treat uncomplicated urinary tract infections[5]. Recently, it has been demonstrated that many human pathogenic bacteria have developed resistance against several synthetic drugs and are causing side effects[6,7]. Medicinal plants are the 'back bone' of traditional remedy. The traditional medicine related to treatment of both human and animal diseases with plant-derived preparations is considered a valuable knowledge for the discovery of new antimicrobial and antifungal drugs[8]. *Aristolochia indica* traditional medicinal plant of India reported to be a stimulant, and febrifuge was used for treatment of snakebites, diarrhoea and intermittent fever. The present study aimed at evaluating the *in vitro* antibacterial activity of aqueous and organic root extract of *A. indica* against *E. coli*.

MATERIALS AND METHODS

Plant Material: *Aristolochia indica* L. twinning plant indigenous to India was collected nearby scrubby forest of Bangalore and was authenticated by taxonomist (Herbarium 565) from Department of Studies in Botany, University of Mysore, Mysore, Karnataka. The roots were thoroughly washed and sundried.

Extract Preparation: The roots (10 g) were homogenized in solvents of Acetone, Butanol, DMF, Distilled water and Ether (Reachem).

Test Organism: *Escherichia coli*, the predominant nonpathogenic facultative gram negative non spore forming flora of the human intestine. The culture was obtained from the Department of Studies in Microbiology, University of Mysore, Mysore and suspension was prepared using double distilled water.

Standard Antibiotics: Ciprofloxacin 30 µg/disc, Nitrofurantoin 300 µg/disc Ofloxacin 5 µg/disc, Pefloxacin 5µg/disc and Sparfloxacin 5 µg/disc (HiMedia) tested against *E. coli*. Further the activities were compared with root extracts of *A. indica*.

Preliminary screening was done by Disc diffusion method[9]. Solidified Nutrient agar was seeded with 0.5ml inoculum by spread plate technique. The discs impregnated separately with all five - root extracts, pure solvents were introduced on seeded agar plate, similarly the standard antibiotics. The plates were incubated overnight at 37ºC. The zone of inhibition were measured and expressed in centimeter. The data were subjected statistically to Post Hoc Scheffe one way test.

Table 1: Descriptive statistics (one way) comparing antibacterial activity of solvent against root extract - *Escherichia coli*

	Mean	Subsets	Std. Deviation
Acetone solvent	0.8	ab	0.18257
Plant extract - Acetone	0.725	ab	0.15
Plant extract - Butanol	0.375	a	0.43493
Plant extract – DMF	0.75	ab	0.05774
Plant extract – Distill water	0.75	ab	0.05774
Plant extract - Ether	1.325	b	0.263
Total	0.7875		0.35054
Butanol solvent	0	a	0
Plant extract - Acetone	0.725	bc	0.15
Plant extract - Butanol	0.375	bc	0.43493
Plant extract – DMF	0.75	bc	0.05774
Plant extract – Distill water	0.75	bc	0.05774
Plant extract - Ether	1.325	c	0.263
Total	0.6542		0.45586
DMF solvent	0.3	a	0.34641
Plant extract - Acetone	0.725	ab	0.15
Plant extract - Butanol	0.375	a	0.43493
Plant extract – DMF	0.75	ab	0.05774
Plant extract – Distill water	0.75	ab	0.05774
Plant extract - Ether	1.325	b	0.263
Total	0.7042		0.41017
Distill water solvent	0	a	0
Plant extract - Acetone	0.725	bc	0.15
Plant extract - Butanol	0.375	ab	0.43493
Plant extract – DMF	0.75	bc	0.05774
Plant extract – Distill water	0.75	bc	0.05774
Plant extract - Ether	1.325	c	0.263
Total	0.6542		0.45586
Ether solvent	0.725	a	0.05
Plant extract - Acetone	0.725	a	0.15
Plant extract - Butanol	0.375	a	0.43493
Plant extract – DMF	0.75	ab	0.05774
Plant extract – Distill water	0.75	ab	0.05774
Plant extract - Ether	1.325	b	0.263
Total	0.775		0.34547

a=minimum activity, b=medium activity, c=maximum activity. *Mean with different subsets is significant from each other.

Table 2: Descriptive statistics (one way) comparing antibacterial activity of standard antibiotics against root extract - *Escherichia coli*

	Mean	Subsets	Std. Deviation
Ciprofloxacin	2.875	c	9.57E-02
Plant extract- Acetone	0.725	a	0.15
Plant extract- Butanol	0.375	a	0.4349
Plant extract- DMF	0.75	ab	5.77E-02
Plant extract- Distilled water	0.75	ab	5.77E-02
Plant extract- Ether	1.325	b	0.263
Total	1.133		0.8676
Nitrofurantoin	1.825	c	0.15
Plant extract- Acetone	0.725	a	0.15
Plant extract- Butanol	0.375	a	0.4349
Plant extract- DMF	0.75	ab	5.77E-02
Plant extract- Distilled water	0.75	ab	5.77E-02
Plant extract- Ether	1.325	bc	0.263
Total	0.958		0.5274
Ofloxacin	2.5	c	8.17E-02
Plant extract- Acetone	0.725	a	0.15
Plant extract- Butanol	0.375	a	0.4349
Plant extract- DMF	0.75	ab	5.77E-02
Plant extract- Distilled water	0.75	ab	5.77E-02
Plant extract- Ether	1.325	b	0.263
Total	1.071		0.7387
Pefloxacin	1.925	c	9.57E-02
Plant extract- Acetone	0.725	a	0.15
Plant extract- Butanol	0.375	a	0.4349
Plant extract- DMF	0.75	ab	5.77E-02
Plant extract- Distilled water	0.75	ab	5.77E-02
Plant extract- Ether	1.325	b	0.263
Total	0.975		0.555
Sparfloxacin	3.3	c	0.1414
Plant extract- Acetone	0.725	a	0.15
Plant extract- Butanol	0.375	a	0.4349
Plant extract- DMF	0.75	ab	5.77E-02
Plant extract- Distilled water	0.75	ab	5.77E-02
Plant extract- Ether	1.325	b	0.263
Total	1.204		1.0187

a=minimum activity, b=medium activity, c=maximum activity. *Mean with different subsets is significant from each other.

RESULTS

On comparison of antibacterial assays of pure solvents against five root extracts, all root extracts exhibited different degrees of antibacterial activity compared to pure solvents (Table 1). Ether extract showed maximum activity and butanol extract least. Acetone, DMF and aqueous extracts activities had uniform impact. Among solvents acetone, ether and DMF exhibited activities with not much difference, butanol and distilled water showed no activity but as extracts their activities were similar to other root extracts. On comparison of standard antibiotics against five root extracts (Table 2) ether extract activity was closer to Nitrofurantoin, then with Pefloxacin and Sparfloxacin, Ciprofloxacin and Ofloxacin activities were higher than ether extract.

DISCUSSION AND CONCLUSION

Escherichia coli as enterotoxigenic (ETEC), enteroinvasive (EIEC) and enterohemorrhagic agents (EHEC) cause lot of effects including major complications like dehydration, shock, vascular collapse, and renal failure even in the most robust human hosts. There are no immunizations available yet to develop a safe and effective vaccine to lower worldwide *E. coli* infection rates[10,11]. Antibiotic resistance is a growing problem some due to overuse of antibiotics in humans. The antibiotics used for treatment are known to cause common side effects like headache, rash, itching, nausea, vomiting, loss of appetite, abdominal pain diarrhoea that is watery or bloody, shortness of breath, running out of breath easily, sudden chest pain or discomfort, wheezing, dry cough or hack; fever, chills, body aches, unexplained weight loss; peripheral neuropathy – numbness, dark yellow or brown urine, Nitrofurantoin can cause serious lung injury[12,13]. The increasing failure of chemotherapies and antibiotic resistance exhibited by pathogenic microbial infections agents have led to the screening of several medicinal plants for their potential antimicrobial activity[14,15]. Plants form the main ingredients of medicines in traditional systems of healing and have been the source of inspiration for several major pharmaceutical drugs. Extracts/drugs prepared from medicinal plants having definite mechanism of action are respected among allopathic physicians. Herbal drugs are of much value in chronic and degenerative ailments of the body. Science has enabled us to process natural substances into pills, tinctures and powders [16].

This preliminary screening activity showed roots of *A. indica* exhibiting antibacterial activity against *E. coli* and its extraction with ether was almost near to nitrofurantoin and pefloxacin activity. Not only organic extracts even aqueous extract has shown the efficacy. Therefore on standardization and purification with required dosage concentration the field promises to use ether/aqueous extracts as a suitable antibacterial agent to treat against *E. coli,* the predominant enteric organism.

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