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• Research Article

Antibacterial efficacy of five medicinal plants against multidrug-resistant enteropathogenic bacteria infecting under-5 hospitalized children

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ABSTRACT

OBJECTIVE: To evaluate *in vitro* antibacterial effectiveness of five medicinal plants used by an Indian aborigine, against 8 multidrug-resistant (MDR) enteropathogenic bacteria isolated from clinical samples of under-5 hospitalized children.

METHODS: Antibiotic sensitivity patterns of eight clinically isolated strains of enteropathogenic bacteria, *Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Salmonella paratyphi, S. typhi, Shigella dysenteriae, S. sonnei* and *Vibrio cholerae* were assessed by disc-diffusion method. Antibacterial activities of 8 solvent-extracts of leaves and bark of five medicinal plants were monitored by the agar-well diffusion method. The microbroth dilution method was used to assess minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Qualitative phytochemical analyses of active plant extracts were carried out.

RESULTS: Ethanol, ethyl acetate and methanol extracts of *Holarrhena antidysenterica* leaf tissue were most effective against 8 MDR pathogens *in vitro*. Similarly, acetone, ethanol and methanol extracts of *Terminalia alata* leaf tissue; chloroform, ethyl acetate and methanol extracts of *Terminalia arjuna* leaf tissue and ethyl acetate, ethanol and methanol extracts of *Paederia foetida* leaf tissue were most effective in inhibiting *in vitro* growth of the 8 MDR enteropathogens. Ethyl acetate and methanol extracts of *H. antidysenterica* bark tissue; acetone, ethanol and methanol extracts of *T. alata* bark tissue and acetone, ethanol and methanol extracts of *T. arjuna* bark tissue were most effective in controlling enteropathogen growth. The minimum inhibitory concentration and minimum bactericidal concentration values of the 3 most antimicrobial leaf and bark extracts from the five plants were in the range of 1.56 to 50 mg/mL.

CONCLUSION: These 5 plants exhibited *in vitro* control over a cohort of 8 enteropathogenic bacterial strains isolated from clinical samples.

Keywords: ethnomedicinal plants; multidrug resistance; enteropathogenic bacteria; antibacterial assay; phytochemical analysis; plants, medicinal

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1 Introduction

Enteropathogenic bacteria are the principal causes of high infant and child (under-5) mortality as well as morbidity in all age groups, especially in aged and immunocompromised patients, in developing countries[1]. This group of bacteria includes *Vibrio cholerae*; its infection can quickly lead to a life-threatening situation due to loss of fluid. Such situations are often addressed by infusion of electrolytes to the blood and the simultaneous antibiotic therapy[2]. However, when the causative pathogen is multidrug-resistant (MDR), the antibiotic therapy is ineffective. Further, enteric infections are often characterized by more than one pathogen such as *Escherichia coli* and *Klebsiella pneumoniae* together. Thus, clinical management of these infections can be complicated and unsuccessful[3].

World Health Organization (WHO) records that more than 10 million under-5 children die annually from pneumonia and diarrhoea, with malnutrition being the underlying cause of disease — Asian and African countries contribute more than half of these cases[4]. The mortality rates of under-5 children in Nigeria, the Indian sub-continent and China are the highest in comparison to all other countries. Among Iranian adults and young adults, fluoroquinolone-resistant diarrhoeagenic *E. coli* (DEC), including enteroaggregative, enteropathogenic, enterotoxigenic and shiga-toxin producing strains, accounted for 21.4% of infections[5]. A recent study from India comprising seven hospitals from New Delhi, Vellore, Bangalore and Ludhiana found that the resistance of enteropathogens to antibiotics was greatest for quinolones, macrolides, aminoglycosides, trimethoprim, β-lactams and sulphonamides of higher generations (in decreasing order)[6]. Further it has been summarized that Gram-negative (GN) enteropathogens (*K. pneumoniae*, *Salmonella typhi*, *E. coli*, *S. paratyphi* A and O1 strain of *V. cholerae*) have the highest levels of drug resistance, individually, to tobramycin, amikacin, and cefotaxime; these bacteria were also found to be extended-spectrum β-lactamase (ESBL) positive in 64%, 54%, 32%, 14% and 25% of cases, respectively, in seven hospitals[6]. A Kenyan report found that diarrhoea was the main cause of morbidity and mortality in children in sub-Saharan Africa[7]. Among Kenyan children treated for diarrhoea, 17.7% (of 651 patients) suffered primarily from infection of *Shigella*, *V. cholerae*, *E. coli* and *Salmonella*. Further, *E. coli*, and *Shigella* species were the bacteria which had 80%–100% resistance to the commonly used antibiotics[7]. A study from Nigeria, on the prevalence of Enterobacteriaceae in diarrhoea patients, described *E. coli* O157 as the causative strain, and found it to be resistant to seven common antibiotics; these drug-resistant diarrhoeagenic bacteria were present in both clinical samples and in water samples[8].

Enteric fever in Cambodian children was dominated by *Salmonella enterica*, which was resistant to chloramphenicol and trimethoprim-sulfamethoxazole[9]. Bangladesh, with large areas of lowlands/swamps, was the epicentre of an enteric fever outbreak caused by serotypes of *S. typhi* and *S. paratyphi* of *S. enterica*, which were resistant to chloramphenicol, trimethoprim-sulfamethoxazole, nalidixic acid and ampicillin[10]. In a 2009 study of Romanian children suffering from diarrhoea, 61 of the 250 patients were infected with DEC[11]. Shigellosis and salmonellosis were the most prevalent enteric diseases in children of Mozambique, and several serotypes were described with resistance to the most commonly used antibiotics, including trimethoprim-sulfamethoxazole in 84% cases[12].

Following WHO recommendations for the inclusion of herbal compounds as complementary or alternative medicine (CAM), we selected five well-known ethnomedicinal plants, *Cassia fistula, Holarrhena antidysenterica, Terminalia alata*, *Terminalia arjuna* and *Paederia foetida* (Figure 1) to evaluate *in vitro* antibacterial effects on a cohort of MDR enteropathogenic bacteria isolated from clinical samples of under-5 children suffering from diarrhoea.

Several studies have reported the use of herbal preparations for the control of infectious bacteria[13–15].

C. fistula, the famous Indian laburnum, is known for its medicinal use in treatment of abdominal tumours, glands, liver, throat burns, constipation, convulsions, diarrhoea, epilepsy, leprosy, general skin diseases and syphilis. It is found throughout tropical Asia, South Africa, East Africa, Mexico and Brazil[16].

The plant *H. antidysenterica* has been used for a wide variety of ailments in traditional medicine of India, including for colic and fever, as a carminative, astringent, lithontriptic tonic, aphrodisiac, cardio-suppressant, diuretic and antihypertensive[17]. In Indian Ayurveda, *Kutajarishta*, a combination of 5 phyto-extracts, including leaves of *H. antidysenterica*, is prescribed to control diarrhoea and dysentery[18]. Its seeds are used as an anti-diabetic drug in several Asian countries. The *in vitro* antibacterial activity of *H. antidysenterica* has been shown, as well as its *in vivo* ability to control diarrhoea, dysentery, hemorrhage, hemorrhoids, amoebiasis and hepatitis[17].

T. alata is a deciduous tree with grey or black, deeply cracked, and rough bark, native to India, and also found in Indo-China, Myanmar and Thailand. The tree normally grows to a height of 30–35 m. Traditionally, the bark of *T. alata* has been widely used in Indian ethnomedicine and Ayurveda for a variety of healing purposes[19]. A decoction of the bark is used as cardiotonic, diuretic and styptic, and also to treat boils, bronchitis, diarrhoea, fever, fractures, haemorrhage, pruritus and ulcers; its gum in particular is used as a laxative. To treat diarrhoea or dysentery, about