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Original Research

Monitoring *in vitro* antibacterial efficacy of *Terminalia alata* Heyne ex. Roth, against MDR enteropathogenic bacteria isolated from clinical samples

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Abstract

Background/ introduction: The employed antibiotic could be ineffective against acute health effect causing multidrug resistant enteropathogenic bacteria. Thus, phytodrugs could be used for its control.
Aims/purpose: Leaf and bark extracts of *Terminalia alata*, an ethnomedicinal plant used for ailments of the human gastrointestinal tract, were assessed for antibacterial efficacy *in vitro,* against eight enteropathogenic, extended spectrum β-lactamase enzyme producing multidrug resistant bacteria.
Materials and methods: Pathogenic bacteria were typified with serial biochemical steps. The double-disc diffusion−synergy test was used for the determination of extended spectrum β-lactamase producers. Sixteen antibiotics were used for determining antibiograms of eight isolated bacteria, using the disc-diffusion method. Antibacterial efficacies, of plant-extracts obtained using eight solvents, were monitored by the agar-well diffusion method. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of plant-extracts using solvents, methanol, ethanol, and acetone were determined by the micro-broth dilution method. Seven qualitative phytochemical tests were done with plant extracts.
Results: Of 16 antibiotics, resistant patterns were recorded with 14 antibiotics for *Enterobacter aerogenes* strains, 13 for *Escherichia coli,* 14 for *Klebsiella* sp., seven for *Salmonella paratyphi*, 15 for *Salmonella typhi*, 14 for two species of *Shigella,* and four for *Vibrio cholerae.* It was found that plant extracts using petroleum ether and n-hexane had the least antibacterial activity. Leaf and bark extracts using methanol, ethanol, and acetone registered the highest antibacterial activities with all these bacteria. The methanolic bark extract recorded MIC values, of 1.56 mg/mL against *E. coli*, *Klebsiella* sp., *S. paratyphi*, *Shigella dysenteriae*, and *Shigella sonnei*; 3.13 mg/mL against *E. aerogenes* and *S. typhi*; and 12.5 mg/mL against *V. cholerae*. The MBC values of the methanolic bark extract were: 12.5 mg/mL against *E. coli, Klebsiella* sp., *S. dysenteriae,* and *S. sonnei*; 25 mg/mL against *E. aerogenes, S. typhi*, and *S. paratyphi*; and 50 mg/mL against *V. cholerae.* Phytochemical analyses of methanolic leaf and bark extracts of *T. alata* confirmed the presence of alkaloids, terpenoids, reducing sugars, tannins, and flavonoids.
Conclusion: The antibacterial efficacy of methanolic crude extracts revealed that leaves and bark of *T. alata* could serve as complementary/ supplementary medicines to control multidrug resistant enteropathogenic bacteria.

Keywords: Antibacterial activity; Enteropathogenic bacteria; Minimum bactericidal concentration; Minimum inhibitory concentration; Phytochemical analysis; *Terminalia alata*

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1. Introduction

Terminalia alata Heyne ex. Roth (Indian laurel, locally *Sahaja,* family Combretaceae) is a deciduous tree with gray or black, deeply cracked rough bark, native to India, and found in Myanmar, Indo-China, and Thailand. The tree normally grows to a height of 30−35 m (Fig. 1). Traditionally, *T. alata* bark has been widely used in Indian ethnomedicine and *Ayurveda* for a variety of purposes. The brew of bark is diuretic, styptic, and cardiotonic; it is used against hemorrhage, ulcers, fractures, bronchitis, diarrhea, fever, boils, pruritus, and diseases of the head; also its gum is used as a purgative.[1] About 3−4 teaspoonfuls of fresh bark juice (about 50 g bark crushed in 10 teaspoonfuls of water and filtered) are taken 3 times a day to cure diarrhea and dysentery.[2,3]

Traditional medicines with plants and other natural products are often the major accessible and affordable means of healthcare needs for marginalized people in the developing countries of Asia, Africa, and Latin America, because of efficacy and low cost. Nonedible plants, used in folk medicines traditionally worldwide, are rich sources of natural chemicals of secondary metabolism, and those are, or could be, formulated for modern medicines for several health conditions.[4] More than 80% of the world's population, often consisting of the elite, relies on plant-based products to meet their healthcare needs; 25−45% of prescriptions today of the modern medicinal system contain plant-derived molecules as basic sources.[5] Prior to the advent of synthetic drugs and antibiotics, phytodrugs in crude extracts have been used for healthcare needs ranging from food supplements and antioxidants to pharmaceutically manipulated intermediates for the synthesis of dovetailed drugs for several diseases, giving way to the system of complementary and alternative medicine (CAM). Explicit information, originally obtained from folklore information across diverse cultures with plants, is being embarked scientifically as CAM, because crude extracts of several plants are continually scaled up, for individual use or as coalesced concoctions against a myriad of ailments, worldwide.[4,6−8] Many phytodrugs, such as morphine, quinine, and digoxin—iconic pure phytochemicals are effective for specific health problems. A survey by the World Health Organization (WHO) found that the following proportions of populations use CAM for healthcare needs yields the following: Chile 71%, Columbia 40%, African nations 80%, China 40%, India 45%, among developing countries; and, Australia 48%, Canada 70%, USA 42%, Belgium 38%, and France 75% for developed countries.[9] Furthermore, annual expenditure on CAM was United States Dollar (USD) 500 million in Malaysia, annually compared to USD 300 million for allopathic drugs. In the USA, Australia, UK, and Canada, the annual use of CAM was estimated at USD 300 million, USD 80 million, USD 2300 million, and USD 2400 million, respectively.[9] The value of plant-based prescribed drugs in 1990 was estimated at USD 15.5 billion, which has been on the rise since then to USD 35 billion, during the past decade.[10,11] Not surprisingly, about 42% of the 25 top-selling phytodrugs marketed worldwide are either directly obtained from natural sources or their products.[12] The Indian herbal market is growing fast and should have attained the level of USD 265 billon per annum by 2012, as conjectured from available trends. In particular, the herbal industry potential has revealed that recently the Indian herbal market size was estimated at USD 130 billion, and more than USD 65 billion of herbal raw materials and medicines were exported by India by 2010.[11] This indicated that herbal trade markets and the use of herbal products must be developing in each country, and are not being systematically recorded in databases. Indeed, herbal products are widely recommended today for health boosting and as preventives by WHO,[13] and in future would be recommended for more specific needs as CAM, if those could be scaled as remedial measures, without any dyslogistic prejudice. Obviously, host-toxicity testing of nonedible plant-products remains an essential corollary in CAM for scientific validation. In India, people from the marginalized section—slum-dwellers and aborigines—as well as the sophisticated, elite population habitually use concoctions of crude phytodrugs such as the *Ayurvedic, Unani*, and *Siddha* systems.[10,14] Similar medicinal systems are also in the cultural practices of people of other countries; eventually, phytotoxicity against hosts is frequently recorded.[15] The developed world also has a renewal of interest towards the use of herbal medicines for health boosting or health conditioning and for treating/preventing several common or rare ailments, despite the ready availability of modern medicine.[9] As pharmacology has been acknowledging medicinal plants as the potential sources of bioactive compounds, crude phytoextracts need be considered logistically.

Fig. 1. *Terminalia alata.*

Intractable bacterial infections cause mortality as terminal diseases, and millions suffer chronically from several long-standing infections. On analysis, it is often found that some drug-resistant bacterium is the causative organism of morbidity. Pathogenic bacteria gain multidrug resistant (MDR) traits due to their simple genomes, and consortia of bacteria in nature help their evolution by exchanges of their genetic materials.[10] Consequently, MDR bacteria emerge, causing diverse morbidity and disproportionate mortality. Obviously, several clinical and social factors contribute to the problem of the emergence of MDR pathogens, as detailed elsewhere.[16] Furthermore, a WHO survey identified a rate of nosocomial