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Srivastava Niraj
Assistant Professor& Research Scholar, Department of Kaumarbhritya/Balroga, IMS, BHU.

Saxena Varsha
Research Scholar, Department of Shalya Tantra, IMS, BHU.

Correspondence:
Niraj Srivastava
Assistant Professor& Research Scholar, Department of Kaumarbhritya/Balroga, FOAy, IMS, BHU.

Antibacterial Activity of Kutaj (*Holarrhena antidysenterica* Linn.) in childhood diarrhea: - *In vitro* study

Srivastava Niraj, Saxena Varsha

Abstract
Herbal medicines are significant and reliable sources for treating various infectious and non infectious diseases. It is well known that infectious diseases account for high proportion of health problem, especially in developing countries. Microorganism has developed resistance to many antibiotics and this have created immense clinical problem in the treatment of infectious disease. *Holarrehena antidysenterica* (Linn.) Wall belonging to family Apocynaceae is considered as one of the most effective remedies for treating gastrointestinal diseases such as dysentery and diarrhea in infectious and non-infectious condition**.** As per Ayurveda, Diarrhoea (Atisara) occurs because of abnormal and less potent digestive power (Agnidosha) and (Ajeerna). The clinical manifestations of Atisara are similar to 'Diarrhoea' in modern medicine which is treated with specific Antibiotics and Antispasmodics. The Classics describe; six types of Atisara viz. Vataj, Pittaj, Kaphaj, Sannipataj, Aamatisar and Raktatisar. The seed extract of the Kutaj has been considered as best anthelmintic and is also used to treat various infectious diseases. To evaluate the scientific basis for the use of the plant seed, the antibacterial activities of extract of the seed was evaluated against some common gram positive and gram negative bacteria. *Holarrehena antidysenterica* (L.) has broad spectrum antibacterial activity and has a potential source of new classes of antibiotics that could be useful for infectious diseases. The phytochemical constituents of the dried powdered plant seed was extracted by using aqueous solvents. The antibacterial activity of the concentrated extract was evaluated by the minimum inhibitory concentration (MIC) values of plant extracts against gram positive and gram negative bacteria by using disc diffusion method. The study clearly indicated that the extract of the Kutaj is highly effective in controlling the E.coli, Shegella, Staphylococcal and Salmonella paratyphi-B pathogens responsible for Diarrhoea.

Keywords: *Holarrhena antidysenterica* (L.), Antibacterial activities**,** aqueous extract, Minimum inhibitory concentration.

1. Introduction
Infectious diseases are the leading cause of death world-wide. Diarrhoea is a common symptom of intestinal disorders and it is a global threat to human health [1, 2]. It is a leading cause of morbidity and mortality, with over 1000 million episodes and over 4 million deaths annually in children under five year of age [3]. The WHO has constituted a diarrheal disease control program, which includes traditional medicinal practices together with the evaluation of health education and prevention approaches [4].
Antibiotic resistance has become a global concern [5]. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens [6]. Complementary system of medicine such as Ayurveda, Siddha, Unanai and Chinese medicine have gained its popularity in recent years [7]. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [8]. Therefore, researchers are increasingly turning their attention to Ayurvedic medicine, looking for new leads to develop better drugs against microbial infections. In this study Kutaj was selected for in vitro antibacterial study. Kutaj is a deciduous laticiferous shrub or can be considered as a small tree. The tree grows up to three meters high. The tree has short stem that has pale bark and several branches It consists certain biochemical constituents namely alkaloids, glycosides, phenolic compounds and tannins. Alkaloids have been reported in the bark, leaf and seeds of the plant. Kutaja or Conessine has been used to treat various health ailments such as piles, colic, dyspepsia, chest affections and as a remedy in diseases of the skin and spleen [9].

According to one study, Kutaj seed and bark is capable to kill free living amoebae and it also kills entamoeba histolytica in the dysenteric stools of experimentally infected kittens and the herb is markedly lethal to the flagellate protozoon [10]. The strong antibacterial activity of the Holarrhena antidysenterica extract inhibits growth of enteropathogenic Escherichia coli (EPEC) bacteria strains. The EPEC strains are notorious for resisting the activities of multiple antibiotic drugs. The effectiveness of Holarrhena antidysenterica in treating diarrhea induced by EPEC strains makes it an effective alternative to conventional antibiotic drugs used for treating dysentery [11]. The medicinal plant could also inhibit formation of bloody stools, a symptom of entero haemorrhagic Escherichia coli (EHEC) infection. [12]. Studies suggest that Holarrhena antidysenterica prevents and treats EPEC infections by prevented bacterial adhesion**.** The anti-adherence effect of the alkaloids of the herb provides a rational basis for treating diarrhea induced by EPEC infection. [13].

Holarrhena antidysenterica WALL (Apocynaceae) commonly known as Tell cherry bark (English), Kurchi (Hindi), is a small deciduous tree with white flowers. This plant has many Sanskrit names, the better known being Kutaja and Kalinga. It grows in the tropical Himalayas and is distributed throughout India [14, 15]. Holarrhena antidysenterica is also effective in treating multi-drug resistant Salmonella infection, which is an important cause of severe enteric diseases worldwide [16]. Conessine or Kutaj possesses some properties that fight effectively against the mycobacterium tuberculi and hence, it can be used treating pulmonary tuberculosis [17].

The aim of this study was to evaluate the antibacterial activity of the seed of *Holarrhena antidysenterica* (aquous extracts) against several Gram-positive and Gram-negative bacterial strains in vitro.

B. Materials and methods

Dried Kutaj seed used for this study was collected from Haridwar (Uttarakhand) and identified and authenticated by Prof. V.K Joshi department of Dravaguna, Banaras Hindu University, Varanasi. An antibacterial activity of crude extracts was studied by the disc – diffusion method by Prof. G. Nath in department of Microbiology, Banaras Hindu University, Varanasi. Disc Diffusion method can be adopted by two sub methods.

- Stoke's method
- Kirby – Bauer method

In routine laboratory modified Kirby-Bauer method is used as suggested by NCCLS (National committee for clinical laboratory services), USA (2000).

Preparation of extract- 50 gram of Kutaj seed was coarsely powdered using a mortar and pestle and further reduced to powder using an electric blender. The powder was transferred into close containers. Powdered air dried plant material was extracted with water. 25 gram powdered plant material was mixed in a conical flask with 100 ml of distilled water and then shaken at 120 rpm for 30 minutes and kept for 24 hrs. Extracts was filtered rapidly through four layers of gauge and then filtration through whatman no-1 filter paper. The resulting filtrates were then concentrated and subsequently lyophilized to dryness and then prepare in syrup form.

Text organisms

All the microbial cultures used for antimicrobial screening were procured from National centre for industrial microorganisms (NCIM), Pune, India. The test organism used are Echerichia *coli* ATCC 25992, Pseudomonas *aeruginosa* TCC 10662, Staphylococcus *aurens*, Klebsiella *pneumonia*, Citrobactor *freundii*, Proteus *vulgaris*, Proteus *mirabilis* Pseudomonas *aeruginosa* (clinical isolate), Salmonella *Typhi*, S. Paratyphi A., S. Paratyphi B, S. Typhimurium and V. cholerae 01 classical.

Preparation of Media

Muller Hilton agar (MHA) was used for this antibacterial study in department of microbiology BHU.

Determination of Minimum inhibitory concentration

The disc diffusion method was used for the determination of minimum inhibitory concentration. In vitro efficacy was expressed in terms of minimum inhibitory concentration (MIC) values of plant extracts. for this study Taken a M.H. plate and growth on the plate with particular organism Pure bacterial isolates were grown on Nutrient agar plate. Bacterial suspension of 106 CFU (colony forming unit) / ml was plated spread on the dry plate of M.H.A by cotton swab Aqueous extract of Kutaj was placed on the plate. Then incubated at 37 ^{0}C for 18hrs. At the end of incubation, inhibition zone formed around the disc were measured with transparent ruler in millimeter and the lowest concentration of extract which is showing inhibition of growth of bacteria was determined. The result are the mean value of triplicate tests repeated three times after every 72 hrs of inhibition at 37 ^{0}C ; data statistically significant at $p<0.05$ MIC (minimum inhibitory concentration).

Result and discussion

MIC of Kutaj beej extract on gram positive and gram negative bacteria at different concentration by disc diffusion method was determined to assess their antimicrobial effect. After incubation the inhibition was observed on Escherichia coli ATCC 25992, Salmonella *Typhi* and Staphylococcus aureus. Rest of the strains were not inhibited by 40mg/ml conc and 80mg/ml of Kutaj beej extract. It may be possible that alcoholic extracts of this drugs and higher concentration of this drug can show inhibitory effect on other organism also.

Antibacterial effect of Kutaja

Sterile double dilution aqueous extract of Kutaja was prepared in sterile distilled water. MIC value for E. coli was 16μg/ml and MIC value for staphylococcus aurens was 40μg/ml in 40 mg/ml solution. MIC value for E. coli was 08μg/ml, MIC value for staphylococcus aureus was 20μg/ml and MIC value for Salmonella *Typhi* **is** 40μg/ml in 80 mg/ml solution**. [Table-1]**

Table-1 [MIC Value of drug on different concentration]

Concentration of aqueous extract	MIC Value for E. coli	MIC Value for Staphylococcus *Aureus*	MIC Value for Salmonella *Typhi.*
40mg/ml	16μg/ml	40μg/ml	…….
80mg/ml	8μg/ml	20 μg/ml	40μg/ml

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