



Invitro Antibacterial activity of Arq e Ajeeb on Eschereschia Hirae, Eschereschia Coli, Pseudomonas Aeruginosa and Streptococcus Mutans

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Abstract

The increase in the use of antibiotics and its resistance towards the micro organisms is a matter of great concern to the public health. Although many herbals drugs have been validated for antimicrobials activity, yet it is the need of the hour to figure out a few more antimicrobials. The Unani system of medicine have number of herbs and formulations which are used in treatment of many infections, one such formulation is Arq e Ajeeb(AA) which was used during epidemics for treatment in condition like Cholera (Haiza), Sil (Tuberculosis), Plague (Taoon), Seasonal fevers, and also it was used as anti inflammatory and analgesic. The ingredients of AA have shown antibacterial; hence AA was tested for its synergistic antimicrobial activity. A volume of 100µl sterile MHB broth for bacteria (*P. aeruginosa*, *E. hirae*, *E. coli*, *S. mutans*) was taken, Test sample of 100% concentration was used with double dilutions ranging from 100 – 0.048828mg. The experiment was repeated with Ciprofloxacin at 0.1% as Standard antibiotic. A growth control (Bacterial suspension + 100µl broth medium) and negative control (only broth medium 100µl) was taken. The plates were then incubated at 32.5±2.5°C for 24 to 48 hours. After incubation, 20µl of working solution of resazurin dye was added to all wells. The plates were wrapped with aluminum foil and incubated for 1hour. The color change was then assessed visually. Any colour change from purple to pink or colorless was recorded as positive (growth). MIC value was taken at lowest concentration at which there was no colour change. AA showed antibacterial activity at concentration 100 to 0.048828mg. MIC for *Eschereschia hirae*, *Eschereschia Coli.*, *Streptococcus Mutans* at 0.048828mg. MIC for *Pseudomonas aeruginosa* at 1.562mg. AA can be used as a better alternative to antibacterial drugs, and it has shown antibacterial activity, hence further invitro antimicrobial on other microbes can be undertaken, and as AA ingredients have shown antifungal and antiviral properties, further studies may be undertaken.

Keywords: AA (Arq e Ajeeb), antimicrobial, culture, *E. hirae*, *E. coli*, *S. mutans*, *P. Aeruginosa*.

Introduction

Arq e Ajeeb is a liquid preparation obtained by mixing Camphor (Kafoor), Menthol (Jauhar-e-Pudina), Thymol (Jauhar-e-Ajwayin), in the ratio 2:2:1¹⁻⁴. AA has been used since ancient times orally in a dose of 2-5 drops with water, and also used locally (application, inhalation).

Therapeutic uses Arq e Ajeeb are Nafakh e Shikam (Flatulence of the stomach), Ghisyan (Nausea), Qai (Vomiting), Sue hazm (Indigestion), Waja-ul-Meda (Stomach ache), Ishal (Diarhoea), Wajaul Fawad (Cardialgia), Haiza (Cholera), Qaulanj (Colic), Nazla (Catarrha), Zukham (Coryza), Lazae Hashrat (Irritation by Insect Bite), Shaiqa (Migraine), Suda (Headache)².

Camphor (*Cinnamomum camphora*): Camphor traditionally obtained through the distillation of the wood of the camphor tree, is a major essential oil component of many aromatic plant species, as it is biosynthetically synthesised; it can also be chemically synthesized using mainly turpentine as a starting material.

Camphor exhibits a number of biological properties such as insecticidal, antimicrobial, antiviral, anticoccidial, antinociceptive, anticancer and antitussive activities, in addition to its use as a skin penetration enhancer⁵.

Menthol (*Mentha arvensis*): Essential oil of partially dried leaves of *Mentha arvensis* is extracted by steam distillation method. From this essential oil, menthol crystals are prepared by chilling at different temperatures⁶. Peppermint oil and menthol have moderate antibacterial effects against both Gram-positive and Gram-negative bacteria⁷.

Thymol (*Trachyspermum Ammi*): Thymol found in oil of thyme, and extracted from Ajwain as a white crystalline substance of a pleasant aromatic odor and strong antiseptic properties antibacterial and antifungal effects. Carvacrol and thymol can inhibit growth of both Gram positive and Gram negative bacteria. These compounds have antifungal and antibiofilm effects⁸.

Material and Methods

Preparation of Arq e Ajeeb: Ingredients: the ingredients were purchased from Sana herbals, Kottigepalya, Bangalore, and were authenticated from FRLHT, Bangalore and there voucher specimen no. is 5563-Kafoor (*Cinnamomum camphora*), 5564-Satte Ajwain (*Trachyspermum ammi*), 5565-Satte pudina (*Mentha piperita*) then AA was prepared by crushing crystals of each ingredient separately and then mixed in an air tight container and allowed to liquefy. Later it was filtered and homogenous transparent liquid was obtained and stored in moisture free bottles².

Outline of the method^{9,10}: The AA-Oil evaluated for antimicrobial activity by MIC method *P. aeruginosa*, *E. hirae*, *E. coli*, *S. mutans* at different conc. ranging from 100 to 0.048828 mg/ml. The MIC value of test substance was compared with the standard antibiotic.

Preparation and Standardization of Stock cultures: A loopful of *P. aeruginosa*, *E. hirae*, *E. coli*, *S. mutans* culture was added in 10mL of sterile saline and total numbers of cells were adjusted to 10⁶ CFU/mL at 620nm in digital colorimeter.

Preparation of resazurin and standard antibiotic solution: The stock resazurin solution was prepared by dissolving 2.7mg in 4mL of sterile saline. Further, working solution was prepared by dissolving 1ml of stock solution in 5ml of sterile saline.

The standard antibiotic i.e., Ciprofloxacin at 0.1% concentration was prepared in sterile distilled water.

Preparation of test sample: Test sample of 100% concentration is used.

Determination of antimicrobial efficacy: Minimum inhibitory concentration by microculture method.

A volume of 100µl sterile MHB broth for bacteria and RPMI for fungi was added to all 96 wells except first three wells of the Microtitre plate A1B1C1 to which only 200µl test product was added. In first three wells (A1B1C1) of plate, 200µl of the test product was added and double diluted till (A12B12C12).

The experiment was repeated for positive control that consists of Ciprofloxacin at 0.1% (Standard antibiotic) in second three wells (D1E1F1). To the wells containing test material, 10µl of microbial suspensions of approximately 10⁶ CFU/ml was added. A growth control (Bacterial suspension + 100µl broth medium) from G1 to G12 and broth control (only broth medium 100µl) from H1 to H12 was kept. The plates were then incubated at 32.5±2.5°C for 24 to 48 hours. After incubation, 20µl of working solution of resazurin dye was added to all wells.

The plates were wrapped with aluminum foil and incubated for 1hour. The color change was then assessed visually. Any colour change from purple to pink or colorless was recorded as positive (growth). MIC value was taken at lowest concentration at which there was no colour change.

Results and Discussion

AA has shown antibacterial activity at all concentrations from 100 to 0.048828mg against *S. mutans*, *E. coli* and *E. hirae*, where as *P. aeruginosa* activity was shown from 100 to 1.562mg. Thereby from this we can interpreted that AA has inhibitory activity against *S. mutans*, *E. coli* and *E. hirae* and *P. aeruginosa*.

Table-1: Antibacterial activity of Arq e Ajeeb.

Sample Name	Sample Code	Conc. tested	MIC (mg)*			
			<i>S. mutans</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>E. hirae</i>
AA oil	RR200319	100 to 0.048828 mg	0.0488	1.562	0.0488	0.0488
Ciprofloxacin	Standard	0.1–0.00000012mg	0.000048mg			

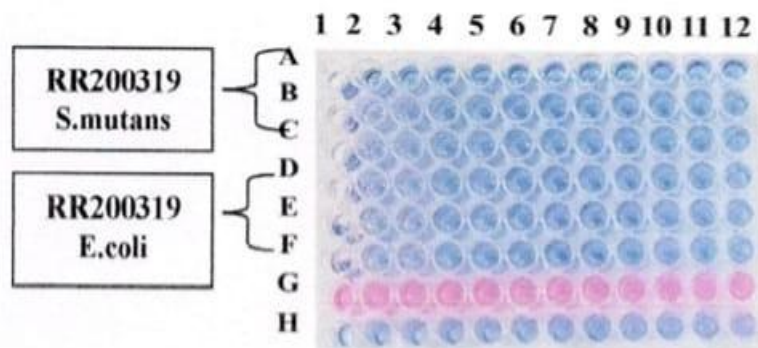


Figure-1: Anti-bacterial activity of *S. Mutans* and *E. coli*. G; Growth control; H; Negative Control.

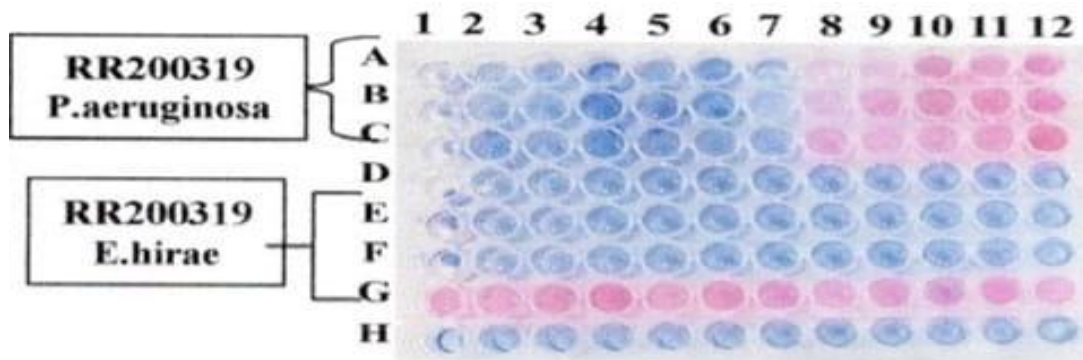
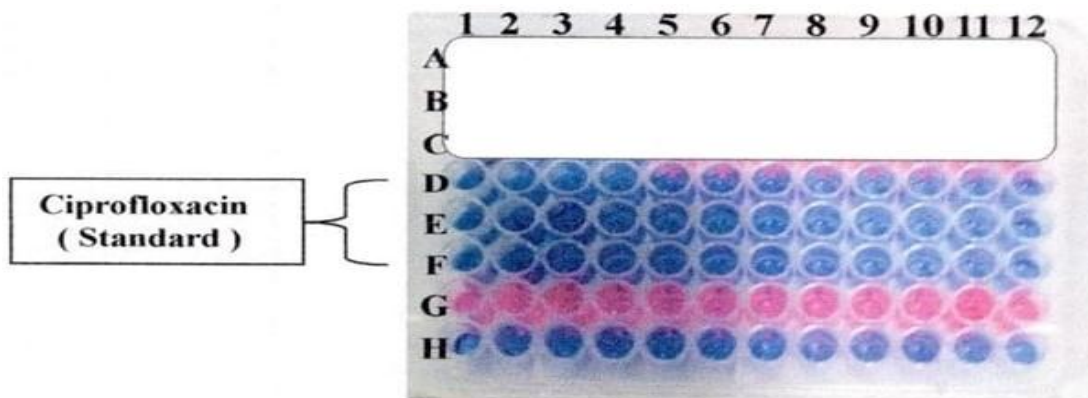


Figure-2: Anti-bacterial activity of *P. aeruginosa* and *E. hirae*. G: Growth control; H: Negative Control.



Note: G: Growth control, H: Negative Control

Figure-3: Anti-bacterial activity of Standard antibiotic against *S. Mutans*, *E. coli*, *P. aeruginosa*, *E. hirae*.

Discussion: As there is increasing risk of multi drug resistance to pathogenic microbes, hence there is quench for search of new antimicrobials which are organic herbal in nature, hence forth a popular unani formulation Arq e ajeeb was initially taken for antibacterial study on two gram positive (*E. hirae*, *Streptococcus mutans*) and two gram negative (*E. coli*, *Pseudomonas aeruginosa*) organism.

In the present study the formulation AA has shown significant inhibitory activity against some pathogenic organisms which causes various diseases.

E. coli causing pneumonia, Urinary tract infection and diarrhea.

E. hirae causing human related pyelonephritis and cholangitis¹¹.

P. aeruginosa mounts infection in weak host defense mechanism; Indeed, *P. aeruginosa* is the opportunistic pathogen of humans. *P. aeruginosa* causes infection in the dermis, soft tissue, bacteraemia, bone and joint, gastrointestinal and blood, urinary tract, respiratory system, particularly in patients with severe burns, tuberculosis, cancer and AIDS. Importantly, *P. aeruginosa* causes a significant problem in patients hospitalized with cancer, cystic fibrosis and burns, with 50% fatality¹².

S Mutans: Streptococcus mutans, are associated with pyogenic and other infections in various sites including mouth, heart, joints, skin, muscle, and central nervous system, *S. mutans* causes dental plaque also¹³.

As we know the mico organisms like *S. mutans*, *E. coli*, *E. hirae*, *P. aeruginosa* are known to cause different ailments respectively in human body, thus a new approach to use Arq e Ajeeb as antibacterial may be considered, however further studies on various organisms may be undertaken to see whether the formulation Arq e Ajeeb has a broad spectrum antibacterial activity.

Discussion

AA Hence it may be considered as safe for internal use with good antibacterial activity with least side effects, which may be used for the benefit of Mankind.

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