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STUDY OF ANTIMICROBIAL ACTIVITY OF AYURVEDIC AND UNANI MEDICINE AND THEIR COMPARATIVE ANALYSIS WITH COMMERCIAL ANTIBIOTICS

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ABSTRACT

The antimicrobial activity of Ayurvedic medicine (Draksharishta, Kanakasava, Jierbadyarishta) and Unani medicine (Alvasin and Carmina syrup) against different microbes (*Staphylococcus* sp., *Escherichia coli*, *Klebsiella* sp., *Salmonella typhi*, *Bacillus subtilis*, *Proteus* sp., *Enterococcus* sp., *Acinetobacter* sp.) were investigated using the agar well method. Ayurvedic medicine were showed more effective antimicrobial activity than unani medicine. In case of Ayurvedic, Jierbadyarishta showed 15mm zone of inhibition against *Proteus* sp., *Enterococcus* sp., *Acinetobacter* sp., *Staphylococcus* sp., *Klebsiella* sp. and 10mm zone of inhibition was observed against *Salmonella typhi*, *Bacillus subtilis* and *Escherichia coli.* On the other hand, Draksharishta showed 15mm zone of inhibition against *Staphylococcus* sp., *Klebsiella* sp., *Salmonella typhi*, *Bacillus subtilis*, *Enterococcus* sp., *Acinetobacter* sp. whereas 17.5mm and 10mm zone of inhibition were found against *Proteus* sp., and *Escherichia coli* respectively. Kanakasava showed 17.5 mm zone of inhibition against *Proteus* sp., *Enterococcus* sp. In all the cases, commercial antibiotic showed less antimicrobial activity than Ayurvedic medicine against all the test organisms. All of Ayurvedic medicine showed the MIC values ranged from 1-100 µl/ml while the MBC values ranged from 0.1-10 µl/ml. This study is an indication that the test Ayurvedic medicine is more challengeable than the test Unani medicine.

KEYWORDS: Ayurvedic, Unani, Antimicrobial, Antibiotic

INTRODUCTION

Ayurveda is a natural system of medicine that has been practiced in India for more than 5,000 years. Ayurveda is a Sanskit word made up of two components, ayush meaning life, and veda meaning knowledge or science. Hence, Ayurveda is the “science of life”. It was developed by seers (rishis) through centuries of observation, experimentation, discussion, and meditation. The origin of ayurvedic medicine are recorded in the Atharva Veda, one of the four Vedic scriptures. For several thousand years, ayurvedic teachings were passed down orally from teacher to student. The first summary of these teachings was put into writing around 1500 B.C. The main sources of knowledge are the three Vedic classics Charaka Samhita, Susruta Samhita, and Ashtanga Hridaya (Mishra *et al.*, 2008). The main objectives of ayurveda are- (i) to maintain and promote health by preventing physical, mental, and spiritual ailments; and (ii) to cure disease through natural medicine, diet, and a regulated lifestyle. In this system of medicine, more than 1200 plants, nearly 100 minerals and over 100 animal products are used. Due to considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics, numerous drugs have entered into international pharmacopoeia.

Ayurvedic medicines are of various types, so as to meet the diverse requirements in the treatment of illness. They are herbal teas, infusions, decoctions, tinctures, capsules and powders, infused oils, ointments, creams, lotions (Kumar *et al.*, 2010). In Bangladesh, there are many ayurvedic Medicine practice now-a-days like Draksharishta, Kanakasava and Jierbadyarishta. Draksharishta is made of grape juice and herbs, but minus the micro-filtration process that modern wineries use, and minus the stringent temperature monitoring in its making and storage. Even now Draksharishta is

prescribed by ayurvedic physicians as medicine, 6 spoons of Draksharishta mixed in equal amount of water, with meals (Chakranews 2011). It improves appetite, relieves constipation, provides strength, induces good sleep and relieves fatigue. It is highly effective in correcting the weakness of lungs, relieving respiratory infection (reduces inflammation of respiratory tract) and reduces cough in tuberculosis patients (Sandu 2012).On the other hand, Kanakasava is mainly used in the treatment of respiratory diseases but should only be taken under strict medical supervision, because it contains a toxic herb ingredient called Dattura. Kanakasava contains 5–10 % of self generated alcohol. The self generated alcohol and the water present in the product acts as a media to deliver water and alcohol soluble active herbal components to the body. It is usually one of the common Ayurvedic medicine for asthma but also used in the treatment of all types of respiratory diseases, COPD, bronchitis, bleeding diseases, injuries and chronic fever, etc. It is a natural mucolytic and bronchodilator. It helps to relieve chest mucous congestion but sometimes it may result in side effects resembling datura poisoning (Ayurmedinfo 2012). Another ayurvedic medicine called, Jierbadyarishta, is a special formulation of several herbs and natural ingredients that helps to control acidity, flatulence and indigestion, that is it tones up the digestive system as a whole and also makes a fine balance between liver and gastric activities.

The Unani System of medicine pioneered in Greece and was developed by Arabs into an elaborate medical science based on the frame work of the teaching of Buqrat (Hippocrates) and Jalinoos (Galen). Since that time Unani medicine has been known as Greco-Arab medicine. This system is based on Hippocratic theory of four humours viz. blood, phlegm, yellow bile and black bile, and the four qualities of states of living human body like hot, cold, moist and dry. They are represented as earth, water, fire and air, the Greek ideas were put by Arabian physician as seven principles (Umoor-e-Tabbiya) and included, element (Arkan), temperament (Mizaj), humours (Akhlat), organs (Aaza), sprit (Arwah), faculties (Qowa) and functions (Afaal). In this system is it believed that, these principles are responsible for the body constitution and its health. (Husain *et al.,* 2010). The World Health Organization (WHO) has recognized the Unani System of Medicine (USM) as an alternative system for the health care needs of human population. Alternative medicine is being practiced worldwide. Unani is one of the most well known traditional medicine systems and draws on the ancient traditional systems of medicine of China, Egypt, India, Iraq, Persia and Syria.

In fact Unani medicine and herbal products are gradually more being used in many countries where modern medicine is easily available. India has accepted it as one of the alternative health care system and has given it official status (Husain *et al.,* 2010). In Bangladesh, there are some unani medicine which includes- Alvasin and Carmina. Alvasin is a unique combination of valuable herbs for all types of cough and cold. Vasicine is the main ingredient of Bashok which has expectorant, bronchodilator, antitussive, mucolytic and antiallergic properties. Jastimadhu has antiallergic properties due to glycyrrhizic acid by inhibiting the secretion of histamin. Alvasin is effective and safe, well tolerated and non-sedative for both adults and children (Hamdard 2008). It is used for all types of cough (dry cough, smokers cough, hacking cough, whooping cough, tubercular cough, allergic cough), common cold, congestion of lungs, bronchitis, tonsillitis, bronchial asthma, hoarseness of voice, sore throat and influenza.

On the other hand, Carmina helps in toning up the mucosa of the stomach and brings about a well tolerated balance between liver functions and gastric activities. Carmina is a special formulation of several natural ingredients that helps to control acidity, flatulence and indigestion (Hamdard 2008). The present study was designed to observe antimicrobial activities of the ayurvedic and unani medicines namely- Draksharishta, Kanakasava, Jierbadyarishta, Alvasin and Carmina. The current study was structured for screening of *in-vitro* antimicrobial activities of collected different samples by agar-well method, application of different concentration of these products against test organisms alone with the comparison against commercial antibiotics, determination of combined effect of antimicrobial activity of these test

products and finally study the effect of antimicrobial activity of these test products against single and mixed microbial culture.

MATERIALS AND METHODS

Throughout the present study, reagent grade nutrient agar medium was used. The selected test medicines were collected from Ayurvedic laboratories Noakhali, Bangladesh and the study was conducted for the period of November 2012 to February, 2013. Both Gram-positive and Gram-negative stains of bacteria (*Bacillus subtilis*, *Enterococcus* sp., *Staphylococcus* sp., *Klebsiella* sp., *Salmonella typhi E. coli*, *Acinetobacter* sp. and *Proteus* sp.) were used as the test organism to observe the antibacterial activity of the test product. These organisms were collected from the Microbiology research laboraroty, Department of Microbiology, Primeasia University, Dhaka, Bangladesh. Test antibiotics in this study are-Tetracycline (30µg/disc), Vancomycin (30µg/disc), Cefaclor (30µg/disc), Sulphamethoxazole / Trimethoprim (25µg/disc), Penicillin (10µg/disc), Clindamycin (2µg/disc), Rifampicin (5µg/disc), Amoxycillin (10µg/disc), Erythromycin (15 µg/disc) and Cloxacillin (5 µg/disc). Agar wells of 5 mm in diameter, 4 mm deep and about 2 cm apart were punched in the NA agar with a sterile cork-borer. Approximately 40 µl of the test product were dropped into each well which filled them respectively to fullness. The setup were allowed to stabilize for 3 h before being incubated at 37°C for 24 h as described previously by Aibinu *et al*. (2007).

Standardization of Inoculum and Assay of Sensitivity Pattern

A standard stock of the bacteria isolates were prepared by suspending a loop full of each microbial growth in about 4 ml of distilled water. After incubation at 37°C for 12 h, the turbidity was adjusted to be visually comparable with a 0.5 McFarland's standard giving a bacterial load of about 3.6×10^2 cfu /mL. 1 ml of each of two different microbial culture (from 3.6×10^2 cfu /mL stock culture) were mixed together aseptically and used for assay the sensitivity pattern against test product.

Determination of MIC and MBC

The MIC is the lowest concentration of the test sample or drug at which it shows the highest activity against microorganism. The minimum inhibitory concentration was determined by serial dilution techniques using nutrient broth media. The minimum inhibitory concentration of test product was determined against all of the selected microbes. Dilutions showing no visible growth for the MIC was sub-cultured and incubated at 37°C for 24h. The lowest concentration of test product yielding no growth on the NA plate was recorded as the MBC. Serial dilutions of test product 1:2, 1:4, 1:8 and 1:16 ml/ml were prepared and their antimicrobial activities were determined by agar well procedure.

Compare with Commercial Antibiotic of the Test Product

Nutrient agar plates were prepared and incubated at 37˚c for 24 h to observed contamination. Then 2 Wells of 5 mm in diameter, 4 mm deep and about 2 cm apart were punched in the middle of the NA agar plate with a sterile cork-borer. Each Nutrient agar (NA) agar plate was uniformly seeded by means of sterile spreader dipped in the suspension and spread on the agar plate surface, and the plates left on the bench for excess fluid to be absorbed. Approximately 40 µl of the test product and 1:2 concentration of the test product were dropped into each well which filled them respectively to fullness. Then ten selected commercial antibiotic disk were placed surround the well at equal distances on NA plate surface by Steriled forceps and incubated at 37˚c for 24h. This procedure was applied for each test product and organism. The antimicrobial activities of the test product and commercial antibiotic were determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

Combined Effect of Test Product against Selected Microbes

Nutrient agar plates were prepared and incubated at 37˚C for 24 h to observed contamination. Then 3 Wells of 5 mm in diameter, 4 mm deep and about 2 cm apart were punched at equal distances on NA agar plate with a sterile cork-borer. Each Nutrient agar (NA) agar plate was uniformly seeded by means of sterile spreader dipped in the suspension and spread on the agar plate surface, and the plates left on the bench for excess fluid to be absorbed. Each well was filled with combination of three of two test product such as one well was filled with combination of 20µl of Draksharishta and 20µl of Kanakasava, another well was filled with combination of 20µl of Draksharishta and 20µl of Jierbadyarishta, and rest one was filled with 20µl Kanakasava and 20µl of Jierbadyarishta. The plates were then incubated at 37˚C for 24h. The antimicrobial activities of the combination of test product were determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

Determination of Effect of Test Product against Mixed Culture

1 ml of stock solution (3.6×10^2 cfu /ml) of each two test microbes were mixed in a sterile test tube. Three Wells of 5 mm in diameter, 4 mm deep and about 2 cm apart were punched at equal distances on NA agar plate with a sterile cork-borer. Then mixture was spread by a spreader on the nutrient agar plate surface. Approximately 40 µl of the test product were dropped into each well which filled them respectively to fullness. The plates were then incubated at 37˚C for 24h. The antimicrobial activities of test product against mixed culture were determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

RESULTS AND DISCUSSIONS

Antimicrobial Activity

Ayurvedic Medicine namely- Draksharishta, Kanakasava, Jierbadyarishta were tested (agar well method) for their antimicrobial activety against all the selected microorganisms. In this antibacterial screening of test product were used at a concentration of 40 µg/well. Unani Medicine namely- Alvasin and Carmina were tested (agar well method) for their antimicrobial activety against all the selected microorganisms. In this antibacterial screening of test product were used at a concentration of 40 µg/well. All of test organisms showed sensitive against all test product (Draksharishta, Kanakasava, Jierbadyarishta). Jierbadyarishta showed 15 mm highest zone of inhibition against *Proteus* sp., *Enterococcus* sp., *Acinetobacter* sp., *Staphylococcus* sp., *Klebsiella* sp. where Draksharishta showed 15 mm zone of inhibition against *Staphylococcus* sp., *Klebsiella* sp., *Salmonella typhi*, *Bacillus subtilis*, *Enterococcus* sp., *Acinetobacter* sp. and 17.5 and 10 mm zone of inhibition against *Proteus* sp., *Escherichia coli* respectively.

Kanakasava showed 18 mm highest zone of inhibition against *Klebsiella* sp. (Table 1). Different concentration of Jierbadyarishta (Neat, 1:2, 1:4, 1:8 and 1:16) were applied in different selected microbes (3.6×10^2 cfu/ml) and the zone of inhibition assayed. In Table 2, it was observed that upto 1:2 concentration showed better result and in the 1:4 the zone of inhibition started to decreased and 1:16 it showed almost negative result.

In case of Kanakasava, it was observed that all the different concentrations alone with Neat showed satisfactory results whereas in case of Draksharishta, satisfactory zone of inhibition were observed only in concentration at neat and 1:2, in the 1:4 the zone of inhibition started to decreased and 1:16 it showed almost negative result (Table 3 & 4). Table 6 showed the antimicrobial activity of Alvasin were 8, 6, 6. 5, 5, 5, 8, 5, and 7 mm zone of inhibition and the antimicrobial activity of Carmina were 7.5, 9, 8, 0, 9, 7.5, 5, and 13 mm zone of inhibition were observed against *Salmonella typhi*, *Proteus* sp., *Acinetobacter* sp., *E.coli*, *Staphylococcus* sp., *Klebsiella* sp., *Bacillus subtilis* and *Enterococcus* sp. respectively.

The results for the antibacterial screening have shown that all tested Ayurvedic Medicines in this present study have antibacterial activity. The results of the inhibition of bacterial growth have shown that tested Ayurvedic Medicines are active at high concentration and inactive at very low concentrations. Thus the study may suggest that the inhibition of bacterial growth activity of the products is dose dependent. It was observed that all the tested Ayurvedic medicines showed remarkable activity against all the tested organisms with the highest activity found on *Klebsiella* sp. (18mm zone diameter) and lowest activity found on *E.coli* (9 mm zone diameter) against Kanakasava at 40µl/well concentration. Jierbadyarishta was also showed almost same antimicrobial activity (15mm) against all the tested organisms. In case of Draksharishta, it showed good antimicrobial activity against all the selected microbes with the highest activity found on *Proteus* sp. (17.5mm zone diameter) and lowest activity against *E.coli* (10 mm zone diameter) at 40µl/well concentration. On the other hand, Tiwari *et al.* (2011) worked on the same (Draksharishta) and found significant reduction in atherogenic index as compared to high fat diet fed control group which strongly supports antiatherosclerotic property of Draksharishta.

Tiwari *et al.* (2011) also showed that Draksharishta and glibenclamide exhibited significant antihyperglycemic activity in alloxan induced diabetic rats without causing significant change in body weight. In this present study it have shown that among all selected microbes, *Proteus* sp. showed highest sensitivity against all the tested Ayurvedic medicines where *E. coli* showed lowest sensitivity. In case of combination of Kanakasava & Jierbadyarishta range of zone of inhibition against the selected microbes was observed from 8-12mm, where 7-10mm zone of inhibition was observed in case of combination of Draksharishta & Jierbadyarishta and 6-8mm zone of inhibition was observed in case of combination of Kanakasava & Draksharishta. On the other hand, in this present study it have shown that Unani medicines (Alvasin & Carmina syrup) have less antimicrobial activity than local Ayurvedic Medicines against all the selected organisms. It was observed that Carmina syrup showed highest antimicrobial activity (13mm zone diameter) against *Enterococcus* sp. and no activity against *E.coli* at 40µl/well concentration. In case of Alvasin syrup, range of zone of inhibition against the selected microbes was observed from 5-8 mm.

Determination of MIC and MBC

In Figure 1, it was observed that the MIC of Jierbadyarishta against *Salmonella typhi, Proteus* sp., *Acinetobacter* sp. and *E. coli* were 1µl\ml whereas MIC against *Klebsiella* sp., *Bacillus subtilis, Staphylococcus* sp., and *Enterococcus* sp. were 0.1µl\ml. The MBC of Jierbadyarishta against *Salmonella typhi* and *Klebsiella* sp. were 10µl\ml and 0.1µl\ml whereas 1µl\ml MBC was observed against all other organisms. In case of Kanakasava, MIC against *Staphylococcus* sp. were 0.1µl\ml and MIC of 1µl\ml were observed in case of other microbes (Figure 2). On the other hand, MBC of 1µl\ml were observed against *Salmonella typhi* and *Enterococcus* sp., 10µl/ml was observed against *Escherichia coli, Proteus* sp., *Acinetobacter* sp. and 0.1µl/ml was observed against all other microbes. In case of Draksharishta, MIC against *Staphylococcus* sp,, *Klebsiella* sp., *Salmonella typhi, Proteus* sp., *Enterococcus* sp. were 0.1µl\ml and MIC of 1µl\ml were observed in case of other microbes (Figure 3). On the other hand, MBC of 1µl\ml were observed against *Klebsiella* sp., *Proteus* sp., and *Acinetobacter* sp.*;* 0.1µl/ml was observed against *Enterococcus* sp., and 10µl/ml was observed against all other microbes.

In present study, it was observed that the MIC of Jierbadyarishta against *Salmonella typhi, Proteus* sp., *Acinetobacter* sp. and *E. coli* were 1µl\ml whereas MIC against *Klebsiella* sp., *Bacillus subtilis, Staphylococcus* sp., and *Enterococcus* sp. were 0.1µl\ml. The MBC of Jierbadyarishta against *Salmonella typhi* and *Klebsiella* sp. were 10µl\ml and 0.1µl\ml whereas 1µl\ml MBC was observed against all other organisms. In case of Kanakasava, MIC against *Staphylococcus* sp. were 0.1µl\ml and MIC of 1µl\ml were observed in case of other microbes. On the other hand, MBC of 1µl\ml were observed against *Salmonella typhi* and *Enterococcus* sp., 10µl/ml was observed against *Escherichia coli,*

Proteus sp., *Acinetobacter* sp. and 0.1µl/ml was observed against all other microbes. In case of Draksharishta, MIC against *Staphylococcus* sp,, *Klebsiella* sp., *Salmonella typhi, Proteus* sp., *Enterococcus* sp. were 0.1µl\ml and MIC of 1µl\ml were observed in case of other microbes. On the other hand, MBC of 1µl\ml were observed against *Klebsiella* sp., *Proteus* sp., and *Acinetobacter* sp.*;* 0.1µl/ml was observed against *Enterococcus* sp., and 10µl/ml was observed against all other microbes. Tiwari *et al.* (2011) observed that Draksharishta inhibited the ferrous sulphate induced lipid peroxidation in a dose dependent manner and showed inhibitory concentration (IC50) value 230.03µg/ml.

Combined Effect of Test Product against Selected Microbes

Combination effect of different test medicines (Kanakasava + Jierbadyarishta, Draksharishta + Jierbadyarishta, and Kanakasava + Draksharishta) showed satisfactory results against all the selected microbes. The zone of inhibition of Kanakasava & Jierbadyarishta were found 8, 11, 12, 11, 9, 10, 10, 8 (Figure 4A) whereas zone of inhibition of Draksharishta & Jierbadyarishta were 10, 10, 9, 9, 7, 10, 9, 10 (Figure 4B) and zone of inhibition of Kanakasava & Draksharishta were 7, 6, 8, 7, 7, 6, 8, 6 (Figure 5) showed against *E.coli, S.typhi, Proteus* sp., *Acinetobacter* sp., *Staphylococcus* sp., *Klebsiella* sp., *B.subtilis* and *Enterococcus* sp. respectively.

Determination of Effect of Test Products against Mixed Culture

In Table 5, Jierbadyarishta was effective against all mixed microbial cultures and the zone of inhibition against *Klebsiella* sp. + *Salmonella* sp., *E. coli* + *Salmonella typhi, E. coli* +*Enterococcus* sp., *B. cereus* + *Staphylococcus* sp., *B. cereus* + *Salmonella* sp., *Enterococcus* sp. +*B. cereus, Proteus* +*Acinetobacter* sp. were found 10, 9, 11, 9, 9, 8, and 10 respectively. In case of Kanakasava, satisfactory results were found against *Klebsiella* sp. + *Salmonella* sp., *E. coli* + *Salmonella typhi, E. coli* +*Enterococcus* sp., *B. cereus* + *Staphylococcus spp., B. cereus* + *Salmonella* sp., *Enterococcus* sp. +*B. cereus, Proteus* +*Acinetobacter* sp. where the zone of inhibition were 12, 10, 12, 11, 8, 12 and 10, respectively (Table 6). In case of Draksharishta, satisfactory results were found against *Klebsiella* sp. + *Salmonella* sp., *E. coli* + *Salmonella typhi, E. coli* +*Enterococcus* sp., *B. cereus* + *Staphylococcus* sp., *B. cereus* + *Salmonella* sp., *Enterococcus* sp. +*B. cereus, Proteus* sp. +*Acinetobacter* sp. where the zone of inhibition were 9, 12, 10, 10, 10, 11 and 9, respectively (Table 6). Table 5 shows that all the test products (Draksharishta, Kanakasava, Jierbadyarishta) showed satisfactory results aginst all mixed microbial cultures (*Klebsiella* sp. + *Salmonella* sp., *E. coli* + *Salmonella typhi, E. coli* +*Enterococcus* sp., *B. cereus* + *Staphylococcus* sp., *B. cereus* + *Salmonella* sp., *Enterococcus* sp. +*B. cereus, Proteus* sp.+*Acinetobacter* sp.*).*

Comparison of Test Medicine Products with Commercial Antibiotics

In case of *E. coli* the test products namely- Jierbadyarishta, Kanakasava, Draksharishta showed 10, 9, and 10 mm zone of inhibition respectively whereas 14, 8 mm zone of inhibition were observed against Sulphamethoxazole/ Trimethoprim, and Erythromycin and respectively. At the time of *S. typhi* 10, 16, 15 mm zone of inhibition were showed against Jierbadyarishta, Kanakasava, Draksharishta respectively whereas 12, 8, and 11 mm zone of inhibition were observed against Sulphamethoxazole/Trimethoprim, Erythromycin and Cloxacillin respectively. In case of *Proteus* sp. highest zone of inhibition was observed 15, 17.5 and 17.5 mm against Jierbadyarishta, Kanakasava, Draksharishta whereas 13, 9, 8, and 9.5 clear zone were observed against Sulphamethoxazole/Trimethoprim, Penicillin, Amoxycillin and Cloxacillin respectively.

At the time of *Acinetobacter* sp. clear zone of inhibition of 15, 17.5 and 15 mm were observed against Jierbadyarishta, Kanakasava, Draksharishta respectively whereas zone of inhibition by Sulphamethoxazole/Trimethoprim, Rifampicin, and Erythromycin were 14, 6 and 7 mm respectively against *Acinetobacter* sp. In case of *Staphylococcus* sp. the test products namely- Jierbadyarishta, Kanakasava, Draksharishta showed 15, 13, and 15 mm zone of inhibition

respectively whereas 17, 6.5, 9 and 8 mm zone of inhibition were observed against Clindamycin, Rifampicin, Amoxicillin and Tetracycline respectively. At the time of *Klebsiella* sp. 15, 18 and 15 mm zone of inhibition were showed against Jierbadyarishta, Kanakasava, Draksharishta respectively whereas 12, 7.5, and 9 mm zone of inhibition were observed against Penicillin, Clindamycin and Rifampicin respectively. In case of *B. subtilis* highest zone of inhibition was observed 10, 12.5, and 15 mm against Jierbadyarishta, Kanakasava, Draksharishta whereas 9, 9, 7.5, 14, 7.5 and 12 clear zone were observed against Sulphamethoxazole/Trimethoprim, Penicillin, Clindamycin, Rifampicin, Amoxycillin and Erythromycin respectively. At the time of *Enterococcus* sp. clear zone of inhibition of 15 mm were observed against Jierbadyarishta, Kanakasava, Draksharishta whereas zone of inhibition by Clindamycin, Rifampicin, Amoxycillin, Tetracycline, Cefaclor and Vancomycin were 14, 9, 7.5, 9, 7.5 and 12 mm respectively against *Enterococcus* sp. (Figure 7 and 8). Satisfactory results were observed in all the tested ayurvedic medicines as compared with commercial antibiotics.

In this present study all of the ayurvedic medicines (Draksharishta, Kanakasava, Jierbadyarishta) tasted showed more effective antimicrobial agent than unani medicine (Alvasin and Carmina syrup) against all the tested organisms in controlling their growth *in vitro* in culture condition. They all have a bacteristatic and bactericidal activity when tested *in vitro* using selected neat sample. In this project it was interestingly found that all of the tested ayurvedic medicines have almost twice antimicrobial activity than unani medicine, even whereas most commercial antibiotic have a little response against all the tasted organisms. The microbes that were selected in this present study can cause different types of diseases. As if the tested ayurvedic medicines were more effective than commercial antibiotics like Sulphamethoxazole/ Trimethoprim, Erythromycin, Tetracycline, Penicillin and they can be successfully used for the treatment of various diseases.

The tested local ayurvedic medicines can also be preferred inested of the area of unani medicine application. More importantly there is need for detailed scientific study of traditional practices to ensure the valuable therapeutic knowledge of tested ayurvedic medicine and also to provide scientific evidence for their efficacies. Since the tested ayurvedic medicines are made from natural plant products which are available in our country sides and having traditional medical use, development of modern drugs can be contributed with ayurvedic composition to cure very common diseases in our country at cheap rate especially for the people of rural areas. In fine the time has come to get turn into ayurvedic medicine application which has no side effects. For the last few years the mass awareness tends to ayurvedic medicine usage and increasing day by day.

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APPENDICES

Table 1: Determination of Zone of Inhibition by Ayurvedic Medicine

Name of Organisms	Zone of Inhibition (mm)		
	Jierbadyarishta	Kanakasava	Draksharishta
Salmonella typhi	10	16	15
Proteus sp.	15	17.5	17.5
Acinetobacter sp.	15	15	15
E. coli	10	9	10
Staphylococcus sp.	15	13	15
Klebsiella sp.	15	18	15
Bacillus subtilis	10	12.5	15
Enterococcus sp.	15	15	15

Table 2: Application of Jierbadyarishta in Different Concentration

Name of Organisms	Zone of Inhibition (mm)				
	Neat	1:2	1:4	1:8	1:16
Enterococcus sp.	15	9	5	5	0
Salmonella typhi	10	7.5	0	0	0
Bacillus subtilis	10	6	0	0	0
Acinetobacter sp.	15	0	0	0	0
E. coli	10	0	0	0	0
Klebsiella sp.	15	5	0	0	0
Staphylococcus sp.	15	4.5	0	0	0
Proteus sp.	15	5	0	0	0

Table 3: Application of Kanakasava in Different Concentration

Name of Organisms	Zone of Inhibition (mm)				
	Neat	1:2	1:4	1:8	1:16
Enterococcus sp.	15	8	6	0	0
Salmonella typhi	16	5	0	0	0
Bacillus subtilis	12.5	7.5	0	0	0
Acinetobacter sp.	17.5	8	0	0	0
E. coli	9	7.5	0	0	0
Klebsiella sp.	18	0	0	0	0
Staphylococcus sp.	13	4	0	0	0
Proteus sp.	17.5	0	0	0	0

Table 4: Application of Draksharishta in Different Concentration

Name of Organisms	Zone of Inhibition (mm)				
	Neat	1:2	1:4	1:8	1:16
Enterococcus sp.	15	10	0	0	0
Salmonella typhi	15	7	5	0	0
Bacillus subtilis	15	6	0	0	0
Acinetobacter sp.	15	7.5	0	0	0
E. coli	10	5	0	0	0
Klebsiella sp.	15	0	0	0	0
Staphylococcus sp.	15	5	0	0	0
Proteus sp.	17.5	4	0	0	0

Table 5: Effect of Test Products against Mixed Cultures

Name of Organisms	Zone of Inhibition (mm)		
	Jierbadyarishta	Kanakasava	Draksharishta
Klebsiella sp. + *Salmonella* sp.	10	12	9
E. coli + *Salmonella typhi*	9	10	12
E. coli +*Enterococcus* sp.	11	12	10
B. cereus + *Staphylococcus* sp.	9	11	10
B. cereus + *Salmonella* sp.	9	8	10
Enterococcus sp. +*B. cereus*	8	12	11
Proteus sp. +*Acinetobacter* sp.	10	10	9

Table 6: Antimicrobial Activity of Unani Medicine

Name of Organisms	Zone of Inhibition (mm)	
	Alvasin	Carmina
Salmonella typhi	8	7.5
Proteus sp.	6	9
Acinetobacter sp.	6.5	8
E.coli	5	0
Staphylococcus sp.	5	9
Klebsiella sp.	8	7.5
Bacillus subtilis	5	5
Enterococcus sp.	7	13

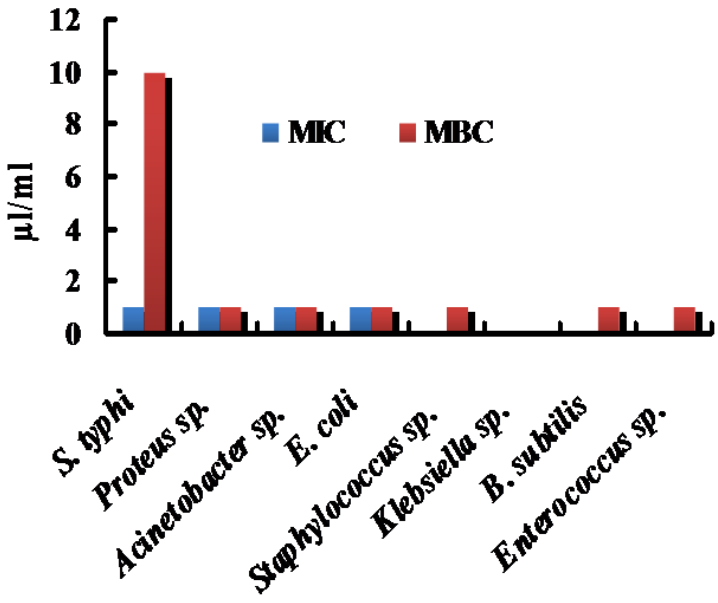

Figure 1: MIC and MBC of Jierbadyarishta

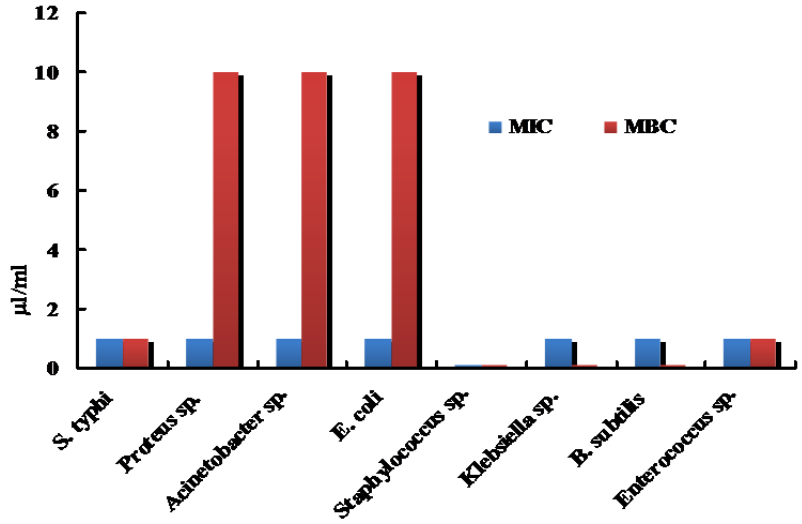

Figure 2: MIC and MBC of Kanakasava

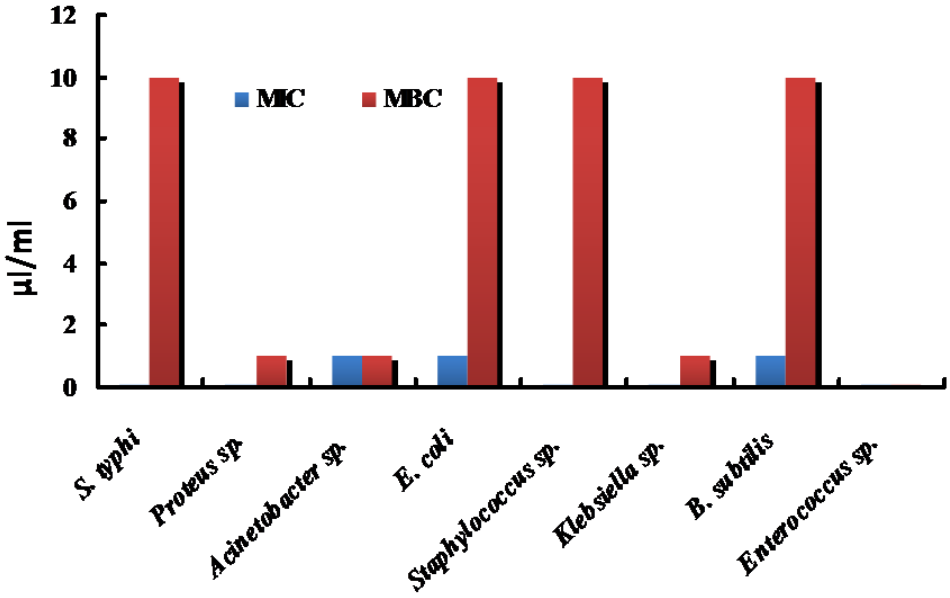

Figure 3: MIC and MBC of Draksharishta

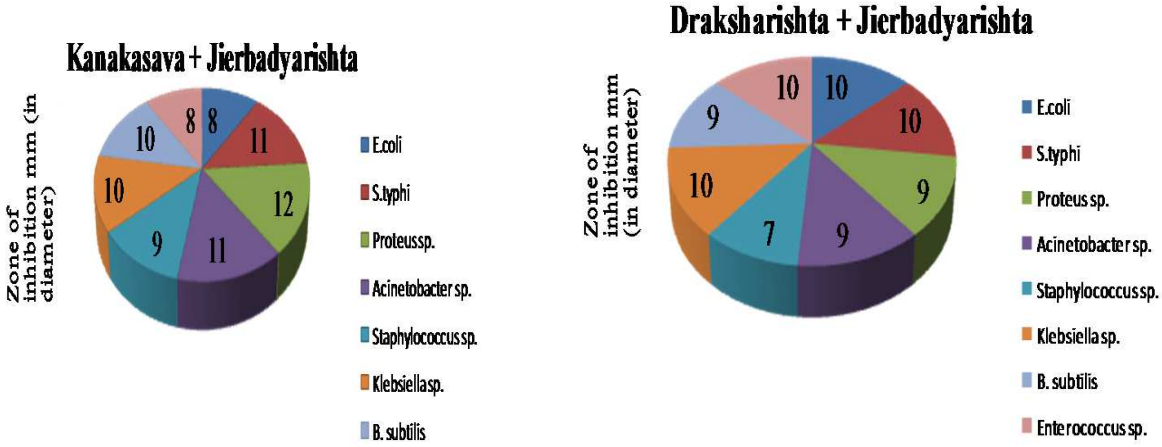

Figure 4: Combined Effect of (A) Kanakasava & Jierbadyarishta (B) Draksharishta + Jierbadyarishta

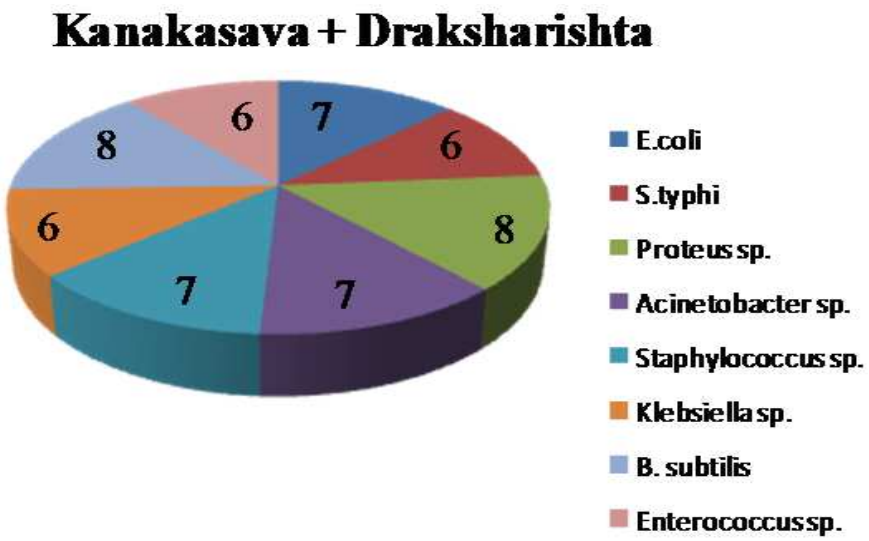

Figure 5: Combined Effect of Kanakasava & Draksharishta

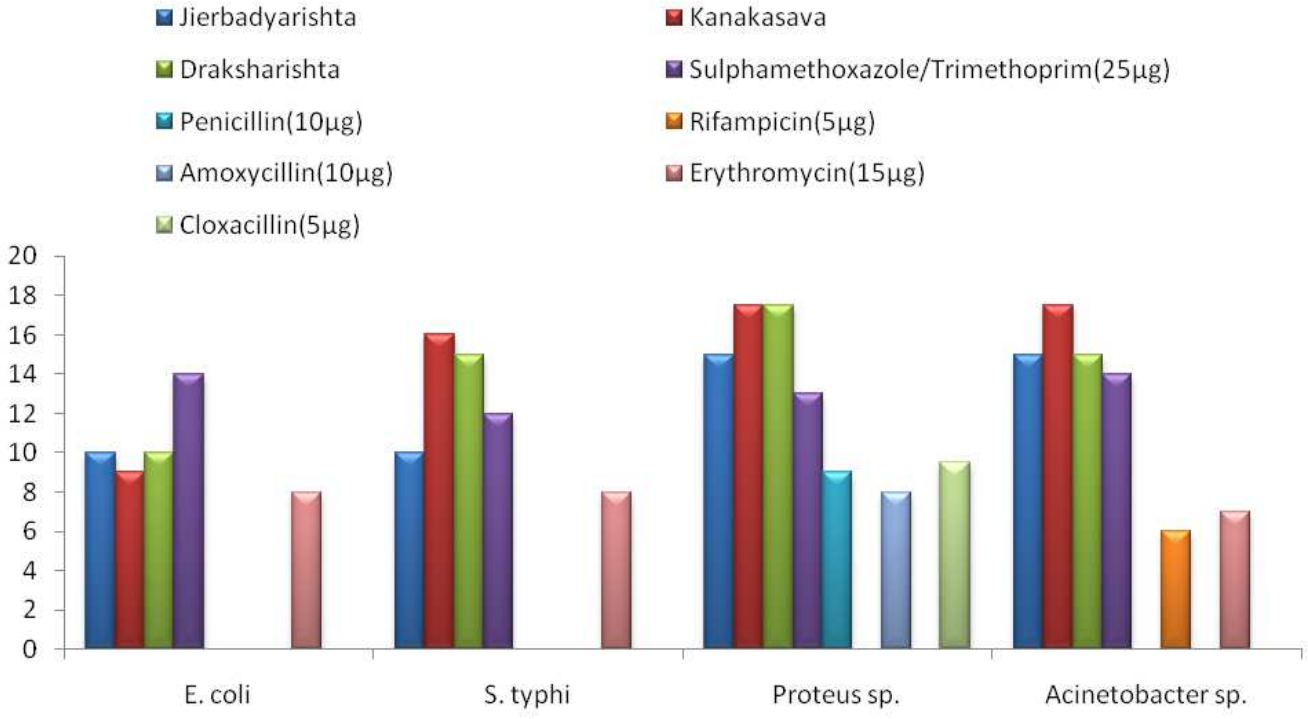

Figure 6: Comparison of Zone of Inhibition of Test Products and Commercial Antibiotics against *E. Coli, S. Typi, Proteus* sp. and *Acinetobacter* sp

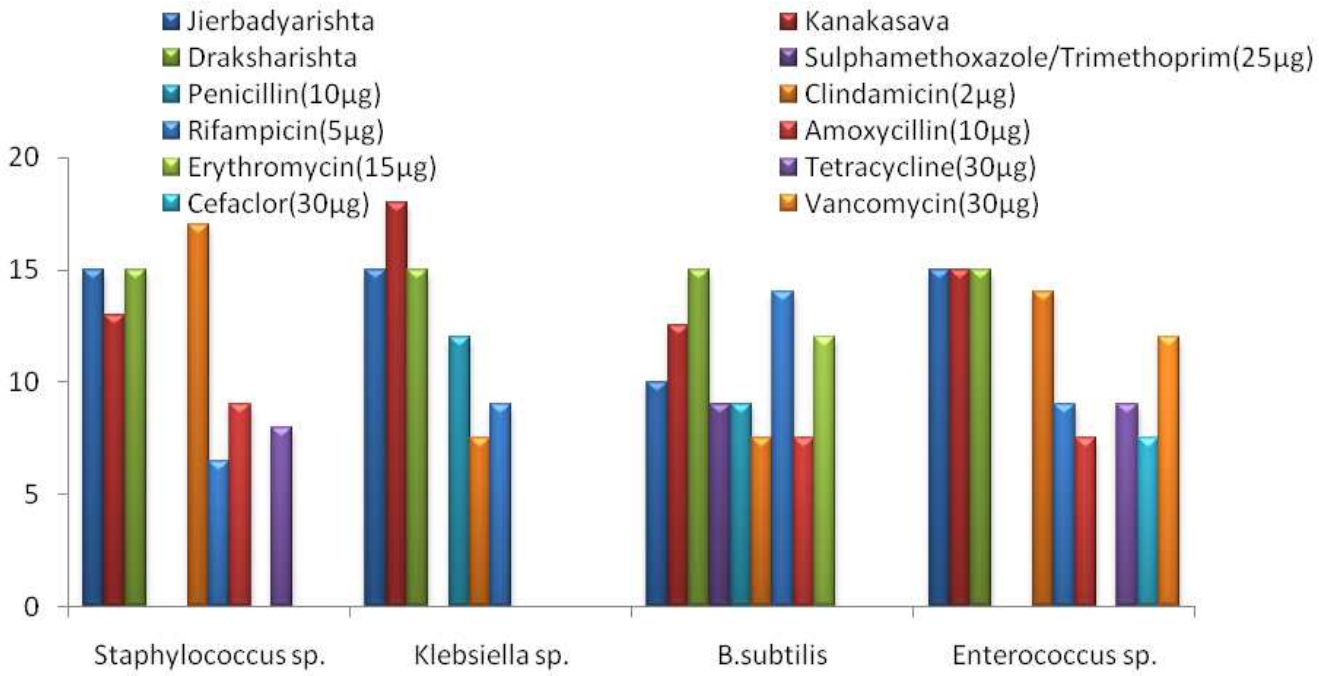

Figure 7: Comparison of Zone of Inhibition of Test Products and Commercial Antibiotics against *Staphylococcus* sp. , *Klebsiella* sp., *B. Subtilis* and *Enterococcus* sp