



ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 8.4
IJAR 2021; 7(10): 240-246
www.allresearchjournal.com
Received: 19-08-2021
Accepted: 21-09-2021

Sabha Shafi
PG Scholar, Department of
Ilmul-advia, Regional
Research Institute of Unani
Medicine, University of
Kashmir, Jammu and
Kashmir, India

Ansar Ahmad
Professor and Head
Department of Ilmul-advia,
Regional Research Institute of
Unani Medicine, University of
Kashmir, Jammu and
Kashmir, India

Mudasir Khazir
Post Graduate Medical Officer,
Department of Indian Systems
of Medicine, Government of
Jammu & Kashmir, Jammu
and Kashmir, India

Afsahul Kalam
Assistant Professor
Department of Ilmul-advia,
Regional Research Institute of
Unani Medicine, University of
Kashmir, Jammu and
Kashmir, India

Mohammad Iqbal Zargar
Senior Assistant Professor,
Department of Pharmaceutical
Sciences, University of
Kashmir, Jammu and
Kashmir, India

Corresponding Author:
Mudasir Khazir
Post Graduate Medical Officer,
Department of Indian Systems
of Medicine, Government of
Jammu & Kashmir, Jammu
and Kashmir, India

Antimicrobial activity of ‘Qurs-afsanteen’: A classical Unani tablet formulation prepared from *Artemisia absinthium*

Sabha Shafi, Ansar Ahmad, Mudasir Khazir, Afsahul Kalam and Mohammad Iqbal Zargar

Abstract

Qurs-afsanteen contains many constituents including *Artemisia absinthium* L. This tablet is being prescribed in various types of fevers, liver diseases, dysuria and malarial fever since decades. Some species of Artemisia herb also contains antimalarial agents and its antimicrobial activities have been evaluated *in vitro*. A plant derived antibiotic may be a possible alternative to modern antibiotics and at the same time can minimize the economic constrain of the hectic process of drug development.

Materials and Methods: The ingredients of the test compound were procured from authentic dealer by Regional Research Institute of Unani Medicine (RRIUM) Srinagar. The dosage form was prepared as per standard procedure in the department of pharmacy, Institute of Asian Medical Sciences Zakura Srinagar. The extract was dissolved in DMSO4 and subject to antimicrobial evaluation at the department of Pharmaceutical Sciences University of Kashmir.

Results: Results obtained in the present study showed that the test drug Qurs-afsanteen possesses promising antimicrobial activity against the selected microbes. The zone of inhibition of the test drug against the *S. typhi* was highest (32millimeters) and good against *E. coli* (20mm).

Conclusion: As the antibacterial activity of the test drug was promising, it may validate the classical claims about use of this drug in case of various infective conditions and further studies are mandated.

Keywords: Qurs-afsanteen, antimicrobial activity, unani medicine, Artemesia absinthium, Dafifuoonat

Introduction

Traditional medicine has been in use since time immemorial and is being practiced in various forms in almost every part of the world particularly Asia and Africa. Traditional medicine mostly uses medicinal plants for this purpose other than mineral and animal origin drugs. Plants are the greatest friends of human beings for various reasons; they have provided us with large number of effective drugs including morphine and many anticancer drugs (Holkova *et al.*, 2019) [11] (Iqbal *et al.*, 2017) [12]. The process of Ufoonat (infection) described in Unani medicine is what we know as infection today. Unani physicians prescribe *Dafi-ufoonat* (anti-infective), *Dafi-bukhar* (antipyretic) and *Muhallil-warm* (anti-inflammatory) drugs for treating various infective conditions.

The indiscriminate use of modern antibiotics not only puts humans to the risk of development of numerous dangerous side effects, but also leads rapid development of antibiotic resistance in bacteria. This process renders the antibiotics useless and also drives the health economy.

The concept of infections is described in Unani medical books as process of “Ufoonat” which literally means putrefaction. Ufoonat requires moisture and heat to develop (Khan., 2011) [17]. There is a long discussion on infective fevers, their causes, nature and pattern of onset, in Unani medical literature (Samarqandi., 2007) [29]. A number of herbs are being used for treating infections of various types and fevers in this system of medicine. The drugs used for this purpose have been classified as *Dafi-ufoonat* (anti-infective), *Dafi-humma* (antipyretic), *Muhallil* (anti-inflammatory) and *Musaffi-dam* (blood purifier). These classes of drugs may be the possible leads for developing herbal antibiotic drugs. Many of them have been proved to have antimicrobial properties in experimental studies (Msaada *et al.*, 2010) [20].

In this study a well-known compound formulation *Qurs-afsanteen* (QA) and was selected for evaluation of antimicrobial activity against four selected bacteria. QA is a compound in tablet form containing *Artemisia absinthium* L. (Afsanteen) as main ingredient. The reason for selecting this compound is due to its use in febrile conditions and as blood purifier in Unani system of medicine. There are many such indications of this medicine which point out to its possible activity against bacteria and fungi. *Qurs-afsanteen* contains many constituents including *Artemisia absinthium* L. as chief ingredient. This tablet is being prescribed in various types of fevers, liver diseases, dysuria and malarial fever (Jeelani., 1995) [14]. Some species of *Artemisia* herb also contain antimalarial agents and its antimicrobial activities have been evaluated *in vitro* (Msaada *et al.*, 2010) [20]. Beneficial effects of QA in infective conditions and various other diseases have been mentioned in many classical books pertaining to Unani medicine (Khan., 1995) [18] (Amrohi., 2006) [2] (Sina., 2010) [31]. After preparing *Qurs-afsanteen* as per standard procedure mentioned in Unani Pharmacopeia. (Kabiruddin., 2008) [16] (Samarqandi., 2006) [33] (Arzani., 2006) [4]. Its hydro-alcoholic extracts was obtained and subsequently tested for antimicrobial activity. The antimicrobial activity of the test drug was evaluated against four microbes, *viz.* *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus Subtilis* and *Escherichia coli*. A combination of gram positive and gram negative bacteria were used so that the test drugs could be evaluated for their possible antimicrobial activities against both classes of bacteria.

Materials and Methods

Plant material: The ingredients of *Qurs-afsanteen* (QA) were procured from authentic dealer by Regional Research Institute of Unani Medicine (RRIUM) Srinagar. *Qurs-afsanteen* was prepared as per standard procedure (Arzani., 2009) [5] and guidelines in the laboratory of department of Pharmacy, Institute of Asian Medical Sciences, Zakura Srinagar.

Identification of ingredients and the compound formulation: The ingredients of the two compounds were identified prior to compound preparation and subsequently the compound formulation was also identified and authenticated by Dr Mohd Afsahul Kalam, Asst. Professor, Department of Ilmu Advia (Pharmacology), RRIUM Srinagar. The specimen of the ingredients and the compound drug was preserved in the photochemistry lab. with voucher number S-2016.

Preparation of Extracts: The ingredients of test formulation *Qurs-afsanteen* (QA) were coarsely powdered in an electric grinder. The powder of each drug was extracted separately in 50% alcohol (hydro alcoholic) with the help of soxhlet's apparatus for 6 hours and then the extracts were dried in oven at 60°C. The extracts were dissolved in DMSO (dimethyl-sulphoxide) to required concentration before experimentation (Azwanida *et al.*, 2015) [6] (Rahman *et al.*, 2013) [26].

Mueller Hinton Agar (MHA): Media was procured from HIMEDIA Mumbai in dehydrated form and reconstituted using distilled water as per directions of manufacturer. The composition of Mueller Hinton agar is as follows (Murray *et al.*, 1983) [22]; Meat infusion from 300g; 2.00gms/ltr Casein;

acidhydrolysate 17.50gms/ltr, Starch 1.50gms/ltr, Agar 17.00gms/ltr.

Mueller Hinton Broth (MHB)

Dimethyl Sulphoxide (DMSO): This solvent was used for making test solutions of the extract and was also used as negative control (Costa *et al.*, 2017) [9].

Normal saline: 0.9% NS was used to make bacterial suspension of test bacteria of all the four strains used in the study.

Distilled Water: Distilled water was used for the preparation of MHA and MHB media.

Test microorganisms: A panel of bacterial strains [bacteria: *staphylococcus aureus* (ATCC 6538P), *Bacillus subtilis* (ATCC 19659), *Eshcheria coli* (ATCC 8739), and *Salmonella Typhimurium* (ATCC 13311) were used in the study. All bacterial strains were obtained from HiMedia Mumbai.

Mc Farland standard solution: For preparing a desired inoculum of 6 cfu/ml it has to be compared with 0.5 Mc Farland standard. This standard was prepared by adding 0.5 ml of 0.048 M BaCl₂ to 99.5 ml of 0.18 M H₂SO₄ (1%v/v) with constant stirring. After preparation the standard was transferred to sterile screw cap tubes of equal size and sealed tightly to prevent loss by evaporation. The standard solution was protected from light and stored at room temperature (Zapata *et al.*, 2015) [36].

Revival of bacteria: Lyophilized bacterial cultures were revived as per the manufacturer's instructions (Anonymous. ATCC. 2015) [2]. Sterile MHB was poured into sterile culture tubes up to 4ml. The bacterial tube was opened under laminar hood. With the help of a cotton bud the bacteria were transferred to the broth medium and kept in incubator at 37 °C for 18 to 20hrs. Bacteria were revived on agar medium also. 20ml of sterile agar medium was poured in petri plate and after cooling down cotton bud was dipped in liquid bacteria and the bud was rubbed on the agar medium in streaking manner. The petri plate was kept in incubator at 37 °C for 18-20hrs. All the process was done under laminar hood.

Preparation of bacterial suspension: The bacterial suspensions were prepared by suspending freshly cultured bacterial strains in sterile normal saline (0.9%). The turbidity of the bacterial suspensions was adjusted to 0.5 McFarland standards on spectrophotometer at a wave length of 625nm. These suspensions were used within 30 min of preparation.

Glass ware: Sterile petri plates of 90mm, sterile culture tubes, sterile inoculating loop, sterile cork borer, funnel, beakers, conical flask, stirrer etc.

Positive control

Gentamicin: The Gentamicin antibiotic discs (SD016) were procured from HIMEDIA containing 10µg in each disc, serve as positive control (10µg of tetracycline).

Agar well diffusion method: The bacterial activity tests were carried out using agar well diffusion assay (Balouri *et al.*, 2016) [8]. After sterilization the tubes were cooled down so that a temperature of medium is reached above 45 to 50 °C and inoculation was done at this temperature. This inoculated medium was poured into sterile petri plates of 90mm to yield a uniform depth of 4mm. The media in petri plates was allowed to solidify under laminar hood. After solidifying the media sterile cork borer of 6mm in diameter was used to form uniform wells into each plate. Each petri plate contained four wells with the 40 micro liter volume of each concentration (10mg/ML, 5mg/ML, 2.5mg/ML and 1.25mg/ML) of hydro-alcoholic extract of Qurs-afsanteen, as such the dose of test drug used in the wells was 400mcg, 200mcg, 100mcg and 50mcg respectively.

The extracts were dissolved in DMSO which also served as negative control. The positive control test discs of gentamycin (10 microgram per disc) and tetracycline (10microgram per disc) was also placed in petri plate. All the petri plates were kept for one hour for pre diffusion of extracts before incubating. After 18 to 20 hours in incubator at 37°C the plates were observed for zone of inhibition. The antibacterial activity of extract was measured by antibiotic scale and compared with the standard against the test microorganisms.

Minimum Inhibitory Concentration (MIC) determination

1. Macro Broth dilution method
2. Agar dilution method

For testing the minimum inhibitory concentration of the test drugs two tests broth dilution and Agar dilution were performed for cross checking the results (Baker *et al.*, 1991) [7].

Macro Broth Dilution Method: The MIC of QA extracts (hydro-alcoholic) were determined by macro broth dilution method (James *et al.*, 1998) [13]. The different concentrations of the extract was prepared in DMSO and then 1ml of each concentration was poured into 5ml sterile Muller Hinton media tubes and then 1 micro liter of each bacterial suspension was poured into the MHB medium and mixed thoroughly. Known inoculum of only positive control in one tube under laminar hood was taken. All the tubes were kept in incubator at 37 degree centigrade for 18 to 20 hours and then MIC was determined. MIC was determined as the lowest concentration of extract inhibiting visible growth of each organism in the tube.

Agar Dilution Method: 10ml of sterile MHA was poured in sterile petri plates under laminar hood and then the plates are allowed to solidify. 1ml of each extract at different concentrations was poured into plates along with 1ml of bacterial suspension.

After pre-diffusion of extracts for 1 hour the plates were kept in incubator for 18 to 20 hours at 37 °C. The MIC was determined by measuring the visible zone of inhibition of micro-organisms at different concentrations after comparing it with the growth at the bacterial spot (Wiegand *et al.*, 2008) [35] (Mukharjee *et al.*, 2009) [21].

Results

Microbial load: The microbial load of the extracts was simply checked by placing the sample drugs on sterile petri plates under Laminar hood and were kept in incubator for 24 hours duration and then examined for microbial contamination. No microbial contamination was shown by any extract after incubation period.

Antimicrobial activity: The antimicrobial activity of the test drugs was carried at the department of Pharmaceutical sciences, University of Kashmir. The agar well diffusion method was employed to test the antimicrobial activity of the test drugs. After preparation of the agar medium as per standard procedure it was inoculated with test bacteria. The inoculated media was poured into sterile petri plates of 90 mm to yield a uniform depth of 4mm. The media in petri plates was allowed to solidify under laminar hood. After solidifying the media sterile cork borer of 6mm in diameter was used to form uniform wells into each plate. Each petri plate contained three wells with the 40 micro liter volume of each concentration (10mg/ML, 5mg/ML, 2.5mg/ML and 1.25mg/ML) of hydro-alcoholic extract of Qurs-afsanteen was used in the dose of 40 micro-liter in each petri plate. The extracts were dissolved in DMSO which also served as negative control. The positive control test discs of gentamycin (10 microgram per disc) and tetracycline (10 microgram per disc) was also placed in petri plate. All the petri plates were kept for one hour for prediffusion of extracts before incubating. After 18 to 20 hours in incubator at 37 °C the plates were observed for zone of inhibition. The antibacterial activity of both extracts and (SU) were measured by antibiotic scale and compared with the standard against the microorganisms.

The extract of the compound was subjected to antimicrobial screening against selected pathogens *viz.* *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis* and *Escherichia coli*. The test drugs showed significant zone of inhibition against the selected microbes (Table 1, 2 and 3).

Qurs-afsanteen showed highest activity against *S. typhi* and *S. aureus* at the concentration of 5mg /ml, and substantial antibacterial activity against *E. coli* and *B. subtilis*. Results obtained in the present study showed that the test drug Qurs-afsanteen possess potential antimicrobial activity against the selected microbes. The zone of inhibition of HAEQA against the tested bacteria (*S. typhi*) showed highest activity (32 mm) with working standard antibiotics Tetracycline and Gentamicin. Also, the zone of inhibition of HAEQA against the test bacteria *E. coli*, showed good activity (20mm) with the working standard antibiotics

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration of the test drug was determined using Macro Broth dilution method and Agar dilution method.

The HAEQA showed MIC of 200mcg against *S. aureus*. The Minimum bactericidal concentration (MBC) of the same drug was calculated as 400 mcg. HAEQA showed MIC of 2.5mg/ML against *S. typhi* and MBC was calculated to be 10mg/ML. MIC of the test drug was only determined for those bacterial strains against which the dug showed good antibacterial activity.



Fig 1: Media plate showing extensive growth of *S. aureus*



Fig 4: Agar dilution method for MIC of HAEQA against *S. aureus*



Fig 2: Agar plate showing inhibition of *S. typhi* by HAEQA



Fig 3: Agar dilution method for MIC of HAEQA against *S. typhi*



Fig 5: Macrobroth dilution method for MIC calculation

Table 1: Antimicrobial activity of Hydro-alcoholic extract of Qurs-e- afsanteen (HAEQA) against the four test bacteria

Test organism	Concentration of test drug(HAEQA) (mg/ml)				Standard drug(positive control) (10µg)	
	1Q	2Q	3Q	4Q	TC	GM
<i>S. aureus</i>	30	27	20	17	36	30
<i>B. subtilis</i>	22	21	18	16	33	30
<i>E. coli</i>	20	18	15	12	25	24
<i>S. typhi</i>	32	32	19	10	27	29

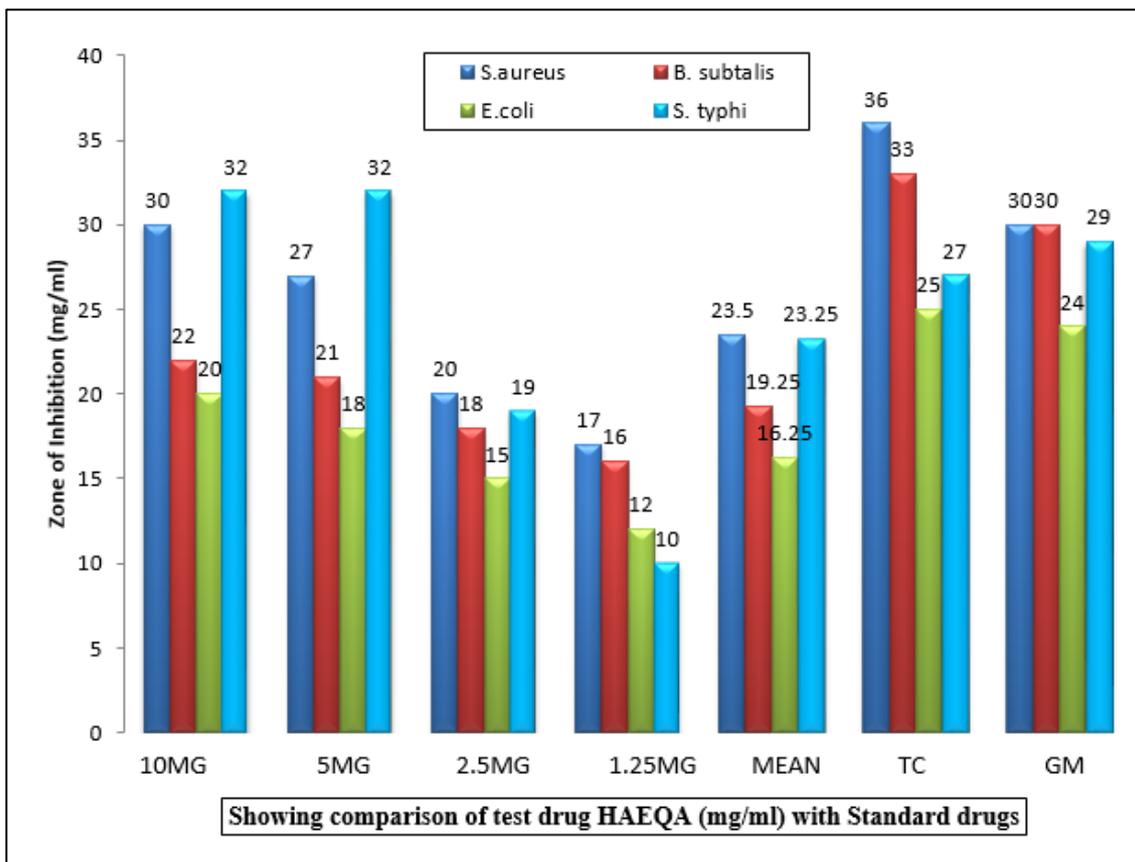
Note: 1Q=10mg/ml (400mcg), 2Q=5mg/ml (200mcg), 3Q=2.5mg/ml (100mcg), 4Q=1.25mg/ml (50mcg) and TC is Tetracycline and GM is Gentamicin

Table 2: Comparison of antibacterial activity of hydro alcoholic extract of Qurs-afsanteen and standard drugs against Staphylococcus aureus

Concentration of test drug (HAEQA) 40µl	Zone of Inhibition (mm)	Zone of inhibition by standard drug	
		Tetracycline (10µg)	Gentamicin (10µg)
10mg/ML	30	37	28
5mg/ML	27	34	32
2.5mg/ML	20	38	30
1.25mg/ML	17	35	30

Table 3: Comparison of antibacterial activity of hydro alcoholic extract of Qurs-afsanteen and standard drugs against *Salmonella typhi*

Concentration of test drug (QA) 40µl	Zone of Inhibition(mm)	Zone of inhibition by standard drug	
		Tetracycline (10µg)	Gentamicin (10µg)
10mg/ML	32	26	28
5mg/ML	32	28	30
2.5mg/ML	19	25	31
1.25mg/ML	10	29	27



TC= Tetracycline: GM= Gentamicin

Fig 6: Showing comparison of activity of Test drug and Standard drugs against different strains of test organisms

It is also worth to note here, that with increase in concentration in test drug and the zone of inhibition tends to increase against all the test organisms used in the study

Discussion

Plants and other natural sources of complex range of chemicals can be promising in development of new drugs. Many researchers focus on identifying leads for drug development from chemicals like oils, saponins, alkaloids, and tannins etc. which occur naturally in plants in a diverse range (Runyoro *et al.*, 2006) [28] (Nazzaro *et al.*, 2013) [24].

There are a number of plant based drugs which show a wide range of antimicrobial activity, including activity against gram positive and gram negative as well as antifungal activities (Cowan *et al.*, 1999) [10]. This fact forms basis for conducting researches in this direction, to continue our search for better and safe alternative antibiotics. Keeping in view the new challenges and trends for developing effective antibiotic substances, some authors suggest to search and develop antibiotic drugs from natural sources. Traditional medicines, including Unani medicine primarily use drugs of natural origin. Therefore they may be able to provide us a lead for developing new antibiotic drugs (Singh *et al.*, 2017) [32] (Rasco *et al.*, 2010) [27].

We are on war with bacteria, while we develop new weapons to destroy them; they form new tactics to protect them. Pathogenic bacteria develop resistance to antibiotics through many ways, either by deactivating the drug or by protecting themselves from effect of the antibiotic drugs on them. Bacterial resistance may be a threat not only to human civilization but animals also, in case new drugs could not be developed against mutating bacteria. In that scenario natural drug sources may either be used on holistic approach basis to aid antibiotics in their action through synergism, or they can be a good alternative source of antibiotic leads owing to presence of vast range of active metabolites in them. Plants have shown antibacterial activity against many pathogens, and also have been shown to act against multidrug resistant strains of NDM-1 group and others. All these findings encourage further studies on natural sources of drugs for development of alternative antibiotic agents (Thakur *et al.*, 2013) [34].

In this study a compound formulation. Qurs-afsanteen (tablet) was selected for evaluation of antimicrobial activity. Qurs-afsanteen (tablet) contains many constituents including *Artemisia absinthium* as chief ingredient. This tablet is being prescribed in various types of fevers, liver diseases, dysuria and malarial fever¹⁹. *Artemisia herb* also contains

antimalarial agents and its antimicrobial activities have been studied in detail (Panseeta *et al.*, 2011) [25].

The test drug under study is a well known antipyretic and anti-infective agent used in Unani medicine. It is commonly indicated in febrile and infective conditions, The anti-infective and blood purifying action may be attributed to the particular *Surat-i-Naw'iyā* of the drug as per Unani theory (Nafees., NA) [23]. A drug can be either directly antipyretic or indirectly by killing or stopping the growth of pathogens in the body which cause fever. Similarly the chemicals responsible for antipyretic effect of the Qurs-afsanteen may be possessing antipyretic or antibiotic effects. As nature has provided plants with lots of chemically different constituents ranging from painkillers to anticancer drugs, our goal is to identify and separate the pharmacologically active constituents from plants traditionally used for different ailments since ages. The concept of infections is described in Unani medical books as process of "Ufoonat" which literally means putrefaction. Ufoonat requires moisture and heat to develop (Majusi., 2010) [19]. There is a long discussion on infective fevers, their causes and nature and pattern of onset, in Unani medical literature (Jurjani., 2010) [15]. A number of herbs are being used for treating infections of various types and fevers in this system of medicine. The drugs used for this purpose have been classified as *Dafi-ufoonat* (anti-infective), *Dafi-bukhar* (antipyretic), *Muhallil* (anti-inflammatory) and *Musaffi dam* (blood detoxifier). These classes of drugs may be the possible leads for developing herbal antibiotic drugs. Many of them have been proved to have antimicrobial properties in experimental studies (Silva *et al.*, 2010) [30].

The hydro-alcoholic extracts of the test drug showed significant zone of inhibition against the selected microbes. It showed highest activity against *Staphylococcus aureus*. Also showed good activity against *Salmonella typhi* justifying its use of the compound in febrile conditions including typhoid. The test drug showed minimum activity against *Bacillus subtilis*, which is a good indication in other sense. The *B. subtilis* bacterium is basically more friendly than pathogenic to humans (Abatenh *et al.*, 2017) [1]. It has been proven to be normal probiotic organism, residing normally in human gut as a nonpathogenic and friendly commensal. As the test drug showed less activity against this strain, it indicated that the use of this drug in humans may not possibly harm the friendly bacteria in the intestines. As the results of the study were encouraging; further studies on the test drug may be conducted to explore fully its antibiotic potential. This would help in identifying the actual drug molecule responsible for its anti-microbial action.

Conclusion

Following conclusions may be drawn from this research work.

The test drug Qurs-afsanteen possesses significant antimicrobial activities.

The activity of HAEQA is highest against *Staphylococcus aureus* and *Salmonella typhi* strains of bacteria.

The MIC of HAEQA ranges between 5 to 10mg/ML against *S. typhi* and MBC of 400µg against the same bacterium.

The test drug showed no activity against *Bacillus subtilis*, indicating that oral intake of this drug may not be disturbing the normal flora of intestines and *B. subtilis* being one of them.

As the antibacterial activity of the test drugs was promising, it validates the classical claims about use of these drugs in case of various infective conditions.

Acknowledgement

Authors wish to show gratitude towards the CCRUM New Delhi for funding the research work and to the Department of Pharmaceutical sciences university of Kashmir for technical assistance they provided.

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