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LAOOQ SAPISTAN KHYAAR SHAMBARI - A UNANI HERBAL FORMULATION

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Abstract: Laooq Sapistan Khyaar Shambari (LSKS) is a poly herbal Unani formulation used in Unani System of Medicine for the treatment of various upper respiratory tract ailments. This formulation was selected to evaluate its probable antibacterial activity by disk diffusion and Broth dilution method against gram positive bacterial strains (*Staphylococcus aureus, Bacillus cereus, Corynebacterium xerosis, Streptococcus mutans* and *Staphylococcus epidermidis*) and gram negative bacterial strains (*Klebseilla pneumoniae* and *Pseudomonas aeruginosa*). Results revealed that the formulation exhibits significant antibacterial activity against gram +ve and gram –ve bacterial strains. It was concluded that the claims of Unani physician for the usefulness of LSKS in upper respiratory tract infections are in consonance as described by them.

Keywords: Anti bacterial, Laooq Sapistan Khyaar Shambari

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INTRODUCTION

Many diseases are being spread in the world by microorganisms and the discovery of microorganisms as the causative agent of many infectious diseases of man naturally created an interest in substances toxic to these organisms. It is well known that infectious diseases account for a high proportions of the health problems in the third world, hence it becomes necessary to search for quick, effective and safe remedies for these health problems and this gave a stimulus to search for new anti-microbial substances. During this course many substances including some of vegetable origin became recognized as antiseptic, as the anti-microbial substances or preparations which are reported in the literature before about 1940 and referred to as antiseptics or disinfectants or by a variety of other terms generally indicative of toxic actions but not recorded as antibiotics [(1).]

Respiratory tract infection (RTI) is one of the most important infectious diseases worldwide. This infection is the leading cause of morbidity and mortality in critically ill patients in developing countries. RTI are usually contracted through air and by direct contact. At present the therapy for community acquired RTI is often empirical, and how to choose an effective antimicrobial agent is a new challenge to the clinicians, as the composition and the resistance to antimicrobial agents of infectious pathogens was changing frequently [(2,3)].

Scientific experiments on the antimicrobial properties of the plant components were first documented in late 19th century [(4)]. In the present study a poly herbal unani formulation '*Laooq Sapistan Khyaar Shambari*' (LSKS) was used to screen for its probable antibacterial activity. Basically 'Laooq' is the Arabic word that means 'Licking'. It was primarily prepared by Jalinoos (Galen 1st Century) . LSKS is a semi-solid compound formulation which is widely used for the upper respiratory tract diseases. Going through the survey of literature it is revealed that an ample data is available regarding the authentification of LSKS to be used in infectious diseases. Although this compound Unani formulation is in use since time immemorial but very little studies are there, that could correlate it scientifically in its clinical uses. Therefore an effort has been made to carry out its antibacterial activity for the validation of claims of Unani phycians.

Hence a comparative study of LSKS of various samples was made to assess the degree of antibacterial effect. In order to standardize and to lay down the standard operating procedures (SOPs) and Pharmacopoeial standards the formulation was prepared in the Saidla Lab. Deptartment of Ilmul Advia Aligarh Muslim University, Aligarh for a comparative study of different samples of LSKS,The different

samples of LSKS undertaken for the study are as under.

Sample Nos.

1) Self prepared sample of LSKS with Sugar
2) Market sample of LSKS from Hamdard (Wakf) Laboratories B/1-2, III Indl. Area, Meerut Road, Ghaziabad (U.P) India
3) Market sample of LSKS from Rex (U&A) Remedies, Pvt Ltd I-55, Site- V, Surajpur Indl Area, Greater NOIDA, Kasna Distt. Gautam Budh Nagar U.P. India

MATERIALS AND METHODS:

The ingredients of the Unani Formulation 'Laooq Sapistan Khyaar Shambari' as mentioned in the National Formulary of Unani Medicine [5] were procured from Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh. The drugs were properly identified with the help of Botanical Literatures available in the Deptartment and the authenticity was confirmed in Pharmacognosy Section in the Department of Ilmul Advia AMU, Aligarh. A herbarium sample of each ingredient was prepared and submitted in the Museum of the Department of Ilmul Advia for the future reference: Tukhm-e-Khatmi (SC-0122/11); Sanna-e-Makki (SC-0123/11); Unnab (SC-0124/11); Maghz-e-Amaltas (SC-0125/11); Sapistan (SC-0126/11); Roghan-e-Alsi (SC-0127/11); Sat-e-Leemu (SC-0128/11) and Shakar-e-Surkh (SC-0128/11)

Method of preparation (LSKS):

Seeds of Sapistaan (*Cordia latifolia* Roxb.) of weight (125 gm) were crushed initially while the other ingredients (in specified quantities as tabulated in table No.1) were soaked in 14 Litres of double distilled water for 12-14 hrs. After that, all the drugs were boiled for 15 minutes and filtered while hot with the help of the muslin cloth to get the extract of drugs. Sugar was added to the extract of these drugs and boiled after adding sat-e-Leemu. Froth that appeared during boiling was removed manually. The mixture was stirred continuously till the content gained the consistency of syrup. Alsi (Linseed) oil was added and the pan was removed from the burner, 0.1% citric acid was further added and finally the preparation was cooled to room temperature and kept at 4-6^{0}C till further use.[6]

This study was carried out in different ways using Kirby Bauer's disk diffusion and Agar well diffusion method against some pathogenic and ATCC (American Type Culture Collection) strains of different Gram positive and Gram negative bacteria. A total volume of 40 µl of test drugs of various samples were used and compared with the standard drug Ciprofloxacin (30µg) for Gram positive and Gentamicin (30 µg) for Gram negative bacteria. Negative control i.e. Di-methyl sulphoxide (DMSO) the solvent was used.

Antibacterial Screening

The antibacterial screening of LSKS was done as per Clinical and Laboratory

Standard Institute (CLSI) Guidelines against a large number of bacterial strains from ATCC (American Type Culture Collection) strains from Himedia Labs, Mumbai, India and clinical isolates obtained from Jawaharlal Nehru Medical College & Hospital, AMU Aligarh. Results were analyzed on the basis of Zone of Inhibition (ZoI) by Kirby Bauer's disk diffusion Method and Agar diffusion Method (CLSI Guidelines, 2000) and Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) [7].

Agar Diffusion Method

A sterilized swab (PW041, Himedia Labs, Mumbai, India) was dipped into the inoculum suspension (10^6 cfu/ ml) is rotated several times and pressed on the inner side of the test tube to remove excess inoculum from it. The dried surface of the nutrient plate (pH 7.2-7.4) was then streaked with the swab three times, turning the plate at 60^0 angles between each streaking with the test bacterium and finally the rim of the plate, so as to ensure even distribution over the entire surface of plate. The inoculum was allowed to dry for 5-15 minutes with lid in place but not more than 15 minutes, so as to allow for any excess surface moisture to be absorbed before applying the drug.[8]

The wells of the equivalent size were then prepared with the help of a cork borer (6 mm in diameter) in the plate at their previously marked sites, as the process also tear the bottom of the agar, the problem was solved by filling it with few µl of the autoclaved molten agar to avoid diffusion of the drug only at the base. The wells so prepared were filled by the drug sample (40µl) in their respective site with the help of a micropipette. The standard antibiotic disks (Himedia Labs, Mumbai, India) were placed on the prepared plates with sterile forceps and pressed properly to make complete contact with the surface of the medium. Later on these plates were incubated at 37^0C for 24 hours placing them at an inverted position. [9, 10].

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is considered as the 'gold standard' for determining the susceptibility of organisms to antimicrobials and are therefore used as a method of precise assessment to determine the MIC of the antibiotic to the organisms concerned [11]. It was determined according to Broth dilution Method in which different dilutions of both the antibiotics was selected such that the concentration that allowed determination of MIC break-points defining susceptible and resistant values was included. The stock dilutions of the drug sample were prepared so that concentrations ranging from 5000 to19.53 µg/ml were obtained from the original stock solution (20 mg/ml) [11].

Minimum Bactericidal Concentrations (MBC)

Minimum Bactericidal Concentrations (MBC) was determined according to Broth

dilution Method [11] as done for the determination of MIC.

Inoculation of Plates

Refrigerated plates were allowed to equilibrate to room temperature. Once the surface of the plate was dry, 10 µl of 10^4 cfu/ml was delivered on each square marked for the specific strain with the help of micropipette test strains and 1 control strain were tested on each agar plate of different concentrations. Final inoculum thus achieved was 10^4 CPU/ spot on the plate. Inoculation spots were allowed to dry with semi covered lids and then plates were incubated at 37°C for 16-20 hours. Dilutions and inoculations were prepared in the same manner as described for the determination of MIC. The control wells in the microtitre plates containing no drug sample were immediately subcultured (before incubation) by spreading a loopful evenly over a quarter of the plate on a medium suitable for the growth of the test organism and incubated at 37^0C overnight. The microtitre plates were also incubated overnight at 37^0C. The MIC was interpreted from the lowest concentration of the drug sample that inhibited the growth of the organisms and recorded as the MIC. Subculture from the wells showing no visible growth was done in the same manner as done from the control well as described above and incubated at 37^0C overnight. The growth from the control well before incubation (which represents the original inoculum) was compared with the test wells; the purpose of the control was to confirm by its MIC that the drug level is correct, whether or not this organism is killed is immaterial. The highest dilution showing at least 99% inhibition was taken as MBC while three other types of subcultures were also seen in some cases. [12, 13, 14]

Statistical Analysis: All the statistical analysis was done using gpaid software, One way ANOVA was done and the post test named Bonferroni: Selected pairs of column with multiple comparison was performed and p-value is <0.05.

RESULT AND DISCUSSION:

Nowadays there is an increase in the ratio of the side effects occurring from the modern antibiotics and further the infecting micro-organisms are developing resistance to these antibiotics used for them, it was the need of the mankind to explore the antimicrobial efficacy of Unani compound formulations against various micro-organisms. Therefore the present study was done to scientifically validate the use of LSKS for its effect in infectious diseases as claimed by the Unani physicians and it was selected on the basis of empirical evidences present in Unani classical literatures. Results of the antimicrobial activity of LSKS were compared while conducting the study and the comparison was done with Standard drug. i.e Ciprofloxacin (SD142, Himedia Labs, Mumbai, India) for gram positive bacteria and Gentamicin (SD170, Himedia Labs, Mumbai, India) for gram

negative bacteria. Another comparison was done with the solvent used i.e. DMSO which was considered as negative control.

Antimicrobial Activity of LSKS (Sample No.1):

It was found that the test drug produces most effective zone of inhibition against *S. aureus* ATCC 29213 (21.3±0.33) followed by *C.xerosis* ATCC 373 (18.3±1.2); *S.epidermidis* ATCC 155 (16.6±0.33); *S.pyrogenes* ATCC 14289 (16.3±1.2); *B.cereus* ATCC 11778 (14.3±0.88) at higher concentration (20µg/ml) which was comparatively higher than inhibitory zone formed at low concentration. However a significant inhibitory activity against each as compared to Inhibitory zone of Ciprofloxacin 26.8±0.20 mm was seen. Whereas the DMSO the negative control used, (the solvent used for dissolving the LSKS) did not exhibit any inhibitory effect in either case all through the study, which signifies that, this solvent does not have any effect on the growth of the bacteria. While it was found to be resistant to *S.mutans* ATCC 25175; *A.bovis* (clinical isolate) used for the screening.

Antimicrobial Activity of LSKS (Sample No.2)

It was found that the test drug produces most effective zone of inhibition against *S. aureus* ATCC 29213 (21.3±0.33) followed by *C.xerosis* ATCC 373 (20.3±0.88); *S.epidermidis* ATCC 155 (18.3±1.20); *S.pyrogenes* ATCC 14289 (16.3±1.2); *B.cereus* ATCC 11778 (16.3±1.20) at higher concentration (20µg/ml) which was comparatively higher than inhibitory zone formed at low concentration. However a significant inhibitory activity against each as compared to Inhibitory zone of Ciprofloxacin 26.8±0.20 mm was seen. Whereas the DMSO the negative control used, (the solvent used for dissolving the LSKS) did not exhibit any inhibitory effect in either case throughout the study, which signifies that, this solvent does not have any effect on the growth of the bacteria. While it was found to be resistant to *S.mutans* ATCC 25175; *A.bovis* (clinical isolate) used for the screening.

Antimicrobial Activity of LSKS (Sample No. 3):

It was found that the test drug produces most effective zone of inhibition against *S. aureus* ATCC 29213 (21.3±0.33) followed by *C.xerosis* ATCC 373 (20.3±0.88); *S.epidermidis* ATCC 155 (18.3±1.20); *S.pyrogenes* ATCC 14289 (16.3±1.2); *B.cereus* ATCC 11778 (16.3±1.20) at higher concentration (20µg/ml) which was comparatively higher than inhibitory zone formed at low concentration. However a significant inhibitory activity against each as compared to Inhibitory zone of Ciprofloxacin 26.8±0.20 mm was seen. Whereas the DMSO the negative control used, (the solvent used for dissolving the LSKS) did not exhibit any inhibitory effect in either case throughout the study, which signifies that, this solvent does not have any

effect on the growth of the bacteria. While it was found to be resistant to *S.mutans* ATCC 25175; *A.bovis* (clinical isolate) used for the screening.

CONCLUSION:

Among gram positive strains tested LSKS possess maximum antimicrobial activity against *S.pyrogenes* and *S.mutans* (MIC 1.25 µg/ml), while a moderate inhibitory antimicrobial activity against *S.aureus*, *B.cereus*, *C.xerosis* (MIC 2.5 µg/ml), and is resistant against *S.epidermidis* (MIC >5.0 µg/ml). Among Gram negative bacterial strains tested LSKS exhibited almost an equivalent antimicrobial activity against *E.coli*, *K.pneuomoniae*, *P.vulgaris* (MIC 2.5 µg/ml), while for *P.aeruginosa* the MIC was 5.0 µg/ml. The potent antibacterial activity so found could be due to various bioactive constituents present in the various ingredients of this Unani Compound formulation which interact in complex ways to produce the needed therapeutic effect as a whole. It can be concluded that LSKS has an effective antimicrobial property probably due to the presence of various pharmacologically active components.This study may form a good basis for further pharmacological and phytochemical investigations and validate the traditional use of this formulation in respiratory tract infectious diseases.

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Table No. 1: Composition of the formulation of LSKS

S.No	Scientific Name	Common Name	Amount Used	Part Used
1.	*Cassia fistula* Linn.	Amaltas	2.5 Kg	Pulp
2.	*Althaea officinalis* Linn.	Khitmi	250 g	Seeds
3.	*Cardia latifolia* Roxb.	Sapistaan	125 g	Seeds
4.	*Cassia angustifolia* Vohl.	Senna-e- Makki	500 g	Leaves
5.	*Zizyphus vulgaris* Linn.	Unnab	125 g	Fruits
6.	*Saccharun officinarum*	Shakar Surkh	500 g	Crystals
7.	*Saccharun officinarum*	Shakar Safaid/sugar	8 Kg	Crystals
8.	*Citrus limonum* Linn.	Sat-e- Leemu	15 g	Salt
9.	*Linum usitatissimum* Linn.	Alsi (Oil)	125 ml	Oil

Table-2 Comparative AMA of different Test Drug Sample of LSKS against different bacterial Strains

S. No.	Test strains	ZONE OF INHIBITION (in mm) expressed as Mean ± S.E.M (S.D)[Probability of error]				
		Concentration (µgm/ml)			Control (DMSO) (50 µl)	Standard (Ciprofloxacin) 30 µgm
		LSKS (Self prepared)	LSKS (Hamdard)	LSKS (Rex)		
1.	*S.mutans* (ATCC 25175)	6.4±0.24 (0.54)***	6.4±0.24 (0.54)***	6.4±0.24 (0.54)***	6.3±0.88 (1.52)	21.6±2.33 (4.04)
2.	*S.epidermidis* (ATCC 155)	18.3±1.2 (2.08)[ns]	18.3±1.20 (2.08)[ns]	20.3±0.3 (0.57)*	6.3±0.33 (0.57)	24.3±2.02 (3.51)
3.	*B. cereus* (ATCC 11778)	14.3±0.88 (1.5)***	16.3±1.20(2.08)[ns]	14.3±0.88 (1.5)***	6.4±0.24 (0.54)	21.2±0.37 (0.83)
5.	*C. xerosis* (ATCC 373)	18.3±1.2 (2.08)[ns]	20.3±0.88 (1.52)[ns]	20.3±0.3 (0.57)*	6.6±1.33 (2.30)	24.3±2.02 (3.51)
6.	*S.aureus* (ATCC 29213)	20.3±0.3 (0.57)*	6.4±0.24 (0.54)***	18.6±0.88 (1.52)**	6.4±0.24 (0.54)	21.2±0.37 (0.83)
7.	*A.bovis* (Clinical isolate)	6.4±0.24 (0.54)***	6.4±0.24 (0.54)***	6.4±0.24 (0.54)***	6.3±0.88 (1.52)	23.6±0.66 (1.15)
9.	*K. pneumonia* (ATCC 15380)	6.4±0.24 (0.54)***	6.4±0.24 (0.54)***	6.4±0.24 (0.54)***	6.6±1.33 (2.30)	14.0±0.54 (1.22)
10.	*P. aeroginosa* (ATCC 25619)	12.0±0.24(0.54)***	6.4±0.24 (0.54)***	6.4±0.24 (0.54)***	6.3±0.88 (1.52)	14.8±0.20 (0.44)

Fig.1 Comparative AMA of different Test Drug Sample of LSKS against different bacterial Strains

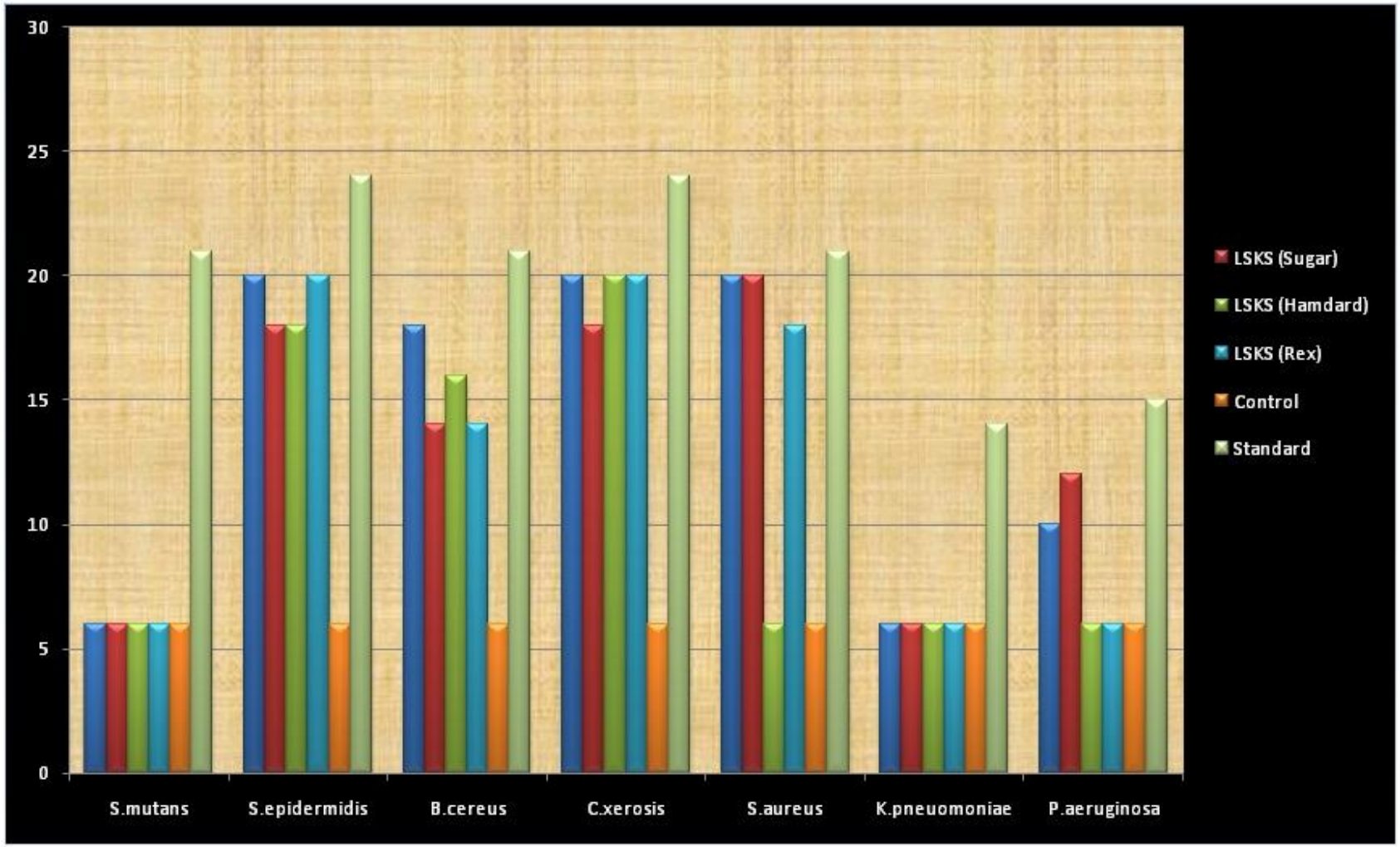

Table-3 MIC and MBC of Test drug sample against micro-organisms (Conc. µg/ml)

S.No.	Strains tested	MIC	MBC
1.	*S. aureus*	2.50	5.00
2.	*S.mutans*	1.25	2.50
3.	*S.epidermidis*	> 5.00	>5.00
4.	*B. cereus*	2.50	5.00
5.	*C. xerosis*	2.50	5.00
6.	*K. pneuomoniae*	2.50	5.00
7.	*P. aeruginosa*	5.00	>5.00

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