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Research Article

ANTIMICROBIAL, HPTLC AND IN-SILICO STUDIES OF HABB-E- BUKHAR - WIDELY USED IN UNANI SYSTEM OF MEDICINE

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ABSTRACT

The Unani system of Medicine, a part of AYUSH, has a detailed documents of drugs "written in classical text" to combat various disorders along with their uses and dosage. Habb-e-Bukhar is one such classical formulation comprising of three ingredients namely *Bambusa Bambos* Druce (Tabasheer), *Cinchona officinalis* L. (Kanakana), and *Tinospora cordifolia* (Willd) Miers. (Satt-e-Gilo). It is said to be given as antipyretics and diaphoretics for acute fever. The drug Habb-e-Bukhar was prepared in laboratory scale using authenticated ingredients. HPTLC fingerprint profile of the drug and its ingredients were analysed to lay down the standards. The aim of present study was to develop HPTLC fingerprints at three different nanometres and evaluate Antimicrobial activity by cup plate method. The mechanism behind the antimicrobial property was studied hypothetically through an *in-silico* analysis. Through an *in-silico* analysis, the interaction between few phyto-compounds of the drug and the organism were studied. Two proteins namely DNA gyrase and Penicillin binding proteins of *Staphylococcus aureus* were targeted for the study. The results reveales good antimicrobial property against all the tested organisms. The *in-silico* analysed data stands as an evidence for the interaction. From the study data, it is perceived that the drug as a whole may yield a synergistic effect in controlling the organism suspected for infection and act as a effective biocompatible anti-therapeutic agent.

INTRODUCTION

Plants have played a unique and holistic role as food, shelter, drugs, clothing etc. Natural compounds have been extensively explored from the plants for new drug discoveries. Phytochemicals revolve around the world as a source of new molecules for pharmaceutical industries leading to the development of novel drugs. Unani medicine is one of the officially recognised traditional medicine system of India which are represented by the word AYUSH i.e., Ayurveda, Yoga, Unani, Siddha and Homeopathy [1]. It has a detailed description of drugs that are utilized for many infectious diseases. Habb-e-Bukhar is a formulated drug given as antipyretics and as diaphoretics for acute fever [2]. It comprises of 3 ingredients namely *Bambusa bambos* Druce. (Tabasheer), *Cinchona officinalis* L. (Kanakana), and *Tinospora cordifolia* (Willd) Miers. (Satt-e-Gilo). Fever may be caused due to many medical conditions [3]**.** In recent years, infections due to bacteria, virus and parasites are common and attributes high rate of mortality. Drug resistance, Multiple drug resistant (MDR) and extremely drug resistant (XDR) infectious pathogens put a greater risk on the population. The bacteria "ESKAPE" pathogens - *Enterococcus faecium, Staphylococcus aureus, Klebsiellla pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species, often develops Multiple Drug Resistance (MDR) and provides major challenge for many pharmaceutical companies worldwide, that scientists claim could return to the level of pre-antibiotic era in designing a potent drug to combat the MDR organisms [4, 5]**.**

The Unani Drug Habb-e-Bukhar comprising the ingredients Tabasheer, Kanakana and Satt-e-Gilo contains several secondary metabolites which are equally efficient to act as anti-therapeutic agents. The genus *Cinchona* (Kanakana) belongs to the family Rubiaceae, consists of nearly 40 species. For commercial purpose three main cinchona species namely, *Cinchona succiruba, Cinchona officinalis* and *Cinchona calisaya* are prevalently cultivated due to the presence of highest alkaloid content in their bark. Among many thousands

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of natural products isolated and characterised so far, the Cinchona alkaloids comprising quinine, quinidine, cinchonidine and cinchonine [6]. Even today it can compete with newer anti-malarial agents, remaining still an important drug for severe malarial cases [7]. *Tinospora cordifolia* (Willd) Miers. (Gilo) commonly called as Guduchi belongs to the family Menispermaceae. The plant is useful in several diseases like jaundice, skin diseases, urinary disorders, gout, diabetes etc., [8-9]**.** and has anti-pyretic, anti-inflammatory and anti-diabetic potential [10]. The water extract, obtained from the stem of Gilo plant is generally referred as "Gilo Satva" or "Satt -e-Gilo" [11]. Apart from starch, the stem of giloy contains, alkaloids - berberine, tinosporin, palmitin etc. [12]**.** Tabasheer, also known as Vanshlochan, bambusha, bamboo manna, eye of the bamboo is the siliceous material found in the culms of some species of bamboo tree e.g. *Bambusa arundinaceae* Retz., *Bambusa bambos* Druce, family Gramineae. It is an extracellular silica found in the stem as a gelatinous mass together with lime and vegetable matter [13]. In India, various types of tabasheer are available in market based on its colour (white, grey, blue) and quality [14]. It is insoluble in water and partially soluble in alcohol. The ingredient Tabasheer can act as nano carrier matrix [15] and in formulations hypothetically can yield synergistic, antagonistic and enhancing properties [13].

The aim of the present study is to evaluate the antimicrobial activity of Habb-e-Bukhar against few medically important organisms. The study extensively elaborates the phyto-constituents of the drug Habb-e-Bukhar through HPTLC fingerprint and the interaction of certain compounds against two target sites DNA Gyrase and PBP'S of the gram-positive organism *Staphylococus aureus* through an *in-silico* molecular docking analysis.

Experimental Section

Method of Preparation

The Unani Formulation - Habb-e-Bukhar was prepared by following the guidelines of National Formulary of Unani Medicine with the ingredients given in Table 1. [2].

Table 1 Ingredients of Habb-e-Bukhar

S.No.	Unani Name	*Botanical Name*	Quantity
1.	Tabasheer	*Bambusa bambos* Druce.	500 gms
2.	Kanakana	*Cinchona officinalis* L.	250 gms
3.	Satt-e-Gilo	*Tinospora cordifolia* (Willd) Miers.	250 gms
4.	Samagh-e-Arabi	*Acacia arabica* Willd.	Q. S.

Note: The formulation may be directly used in powder form (Sufoof) or tablet form (Habb) by adding the excipient - Samagh-e-Arabi filtrate.

Collection of Raw drugs

Raw drugs Tabasheer, Kanakana and Satt-e-Gilo were procured from the local market (RN Rajan stores) Chennai and were authenticated in the Pharmacognosy section of Drug Standardisation Research Unit (DSRU), Regional Research Institute of Unani Medicine (RRIUM), Chennai. The formulation was prepared at laboratory scale in the Department of Chemistry, DSRU, with utmost care by adopting good manufacturing practices as prescribed by the European Guidelines [16]. The voucher specimens were submitted in the Drug Museum, RRIUM, Chennai.

Preparation of the formulation

Sufficient quantities of all the ingredients (1 to 3) were taken, cleaned and kept separately. Tabasheer was finely powdered using mortar with pestle, sieved through 100 mesh and kept aside. Sufficient quantity of ingredient No.2 (Kanakana) was cleaned, shade dried, powdered, sieved through 100 mesh and kept separately. Satt-e-Gilo (powder form) was gently smashed using mortar with pestle and stored separately. The formulation in Sufoof form was made by mixing the required quantities (Table 1) of all the separately grinded powders together without lumps in a wide sterile pan and stored in an air tight container for further use.

Tablet Making (Habb)

For making Habb (tablet form), 125 gms of ingredient No. 4 (Samagh-e-Arabi) was soaked in 750 ml of water (Table 1) and left for 1 hr for complete soaking. The mucilage obtained was squeezed and filtered using clean dry muslin cloth. The filtrate obtained was added to the mixed powders little by little in a sterile wide pan to get the correct consistency of the lubdi mass (dough). Lubdi mass was manually made into Habb of definite shapes (Tablet form) each measuring 250-500 mg. The Huboobs were shade dried and stored in a sterile air tight container for further use.

TLC/HPTLC Finger print Analysis

The prepared formulation (Tablet) and their ingredients Kanakana, Satt-e-Gilo and Tabasheer were extracted separately with 20 ml each of the solvent alcohol, refluxed on water bath for 30 mins and made upto 10 ml in a standard volumetric flask. The extracts formulation (8µl), ingredients, Kanakana (8µl), Satt-e-Gilo (20µl) and Tabasheer (20µl) were applied over aluminium plate pre-coated with silica gel 60 F_{254} (10x10cm, E.Merck) by employing CAMAG ATS4 sample applicator. The plates were developed to a distance of 8 cm in the chamber (20x20cm) using 20 ml of the developing system *Toluene: Ethyl acetate: Formic acid (7.0: 3.0: 0.4)* as mobile phase, dried at room temperature observed and scanned under 254 nm and 366 nm. Finally, the plates were dipped in *vanillin sulphuric acid reagent* (200 ml) for a minute and heated at 105ºC till coloured spots appear and scanned under 540 nm [17].

Collection of microbial cultures for antimicrobial activity studies

The microbial cultures - *Bacillus cereus*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* were collected from Excellent laboratories, Chennai. The cultures were further confirmed using staining techniques and biochemical studies in the Microbiology section, RRIUM, Chennai [18].

Extraction of formulation for Antibacterial activity

For the performance of antibacterial activity, Habb-e-Bukhar in powdered form was used. The formulated drug (2500 mg) was coarsely powdered under sterile condition and soaked in sterile Dimethyl sulfoxide (DMSO -10 ml) for few hours and dissolved to get the stock solution of 250 mg/ml. The drug was serially diluted to get the concentration of 25mg/ml, 2.5 mg/ml, 0.25 mg/ml and 0.025 mg/ml to determine the Minimum Inhibitory Concentration (MIC).

Antimicrobial assay and Detection of MIC

The *in-vitro* antimicrobial activity test and MIC for the formulated drug was performed by cup plate method as per standard methods [19].

The required amount of Muller Hinton agar plates were prepared and swabbed with different isolates of lag phase cultures of organisms namely *Bacillus cereus*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans.* The plates were allowed to stand for few minutes. Required number of 6 mm diameter wells were made over the plates at an equidistant position. Each well was loaded with 75 µl of drug. The Ampicillin disc (10 mcg) was used as standard for comparison. The plates were all kept at 37 ºC for 24 hrs. The zone of inhibition was measured. The MIC was determined to find the lowest concentration of the drug required to completely inhibit the growth of the organisms [19].

Molecular docking studies

To have a better understanding about the inhibitory action and the mode of interaction of phyto-constituents of the formulation against the bacterial organism, docking analysis was performed against the organism *S. aureus.* Two proteins, Penicillin binding protein and DNA Gyrase of *S. aureus* were targeted for the study [20]. The structures DNA Gyrase (3G7B) and PBP'S (3VSL) were retrieved from the protein data bank. The structure of phyto-components of Kanakana (Chinchonine, Chinchonidine, Quinine, Quinidine, Dihydroxyquinidine), Satt-e-Gilo (Berberine, Tinosporin) and Tabasheer (Silica and Silicondioxide) were retrieved from PubChem [6, 21, 22].

Receptor-ligand docking

Autodock Vina (V1.1.2) which is a molecular docking and virtual screening application was used to predict the binding affinities. It is a top-ranking molecular docking application which possess the best scores.

RESULTS AND DISCUSSIONS

Formulation

The drug Habb-e-Bukhar was prepared in a laboratory scale (1 kg). The voucher number obtained for Kanakana (154), Satt-e-Gilo (155) and Tabasheer (156) respectively. The ingredients and the formulation were shown in the Figure 1 (A, B, C and D).

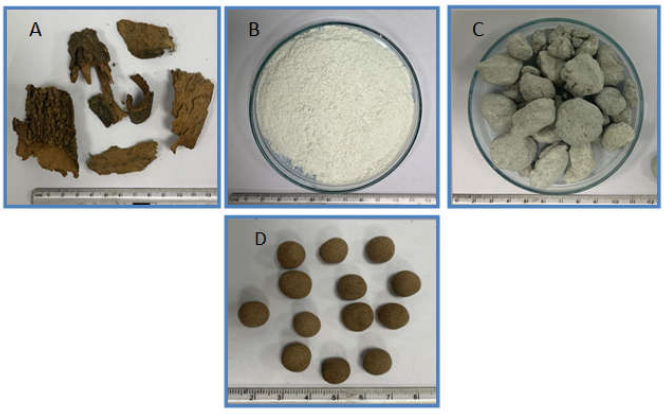
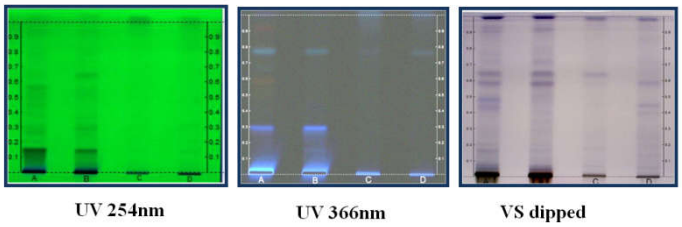


Figure 1 Formulation Ingredients

Kanakana (A) ; Satt-e- Gilo (B) ; Tabasheer (C) ; Habb-e-Bukhar (D)

TLC Finger print

Several developing systems were tried to reach the optimum resolution of the four components. Finally, a developing system consisting of Toluene: Ethyl acetate: Formic acid in the ratio of (*7.0: 3.0: 0.4* ml) was used which resulted in a well resolved peaks. The single drug (ingredients) Kanakana (Track 2) Satt-e-Gilo (Track 3) and Tabasheer (Track 4) served as a marker component for the formulation-Bukhar (Track 1) Figure 2.



Solvent System: Toluene: Ethyl acetate: Formic acid (*7.0: 3.0: 0.4*) ml
Track A: Formulation (8 µl); Track B: Kanakana (8 µl);
Track C: Satt-e-Gilo (20 µl); Track D: Tabasheer (20 µl)

Figure 2 The TLC photographs of alcohol extract of the formulation and its ingredients

HPTLC Fingerprint Analysis

The TLC image obtained was scanned at three different wavelengths viz., 254 nm, 366 nm and 540 nm. The densitometry chromatogram gives the detailed evidence for the presence of the ingredients in the formulation (Figure 3 & 4). On comparison, it is evident that majority of phyto-constituents of Kanakana are present in the formulation followed by Satt-e-Gilo. On the other hand Tabasheer contains only siliceous substances. The R_f values of the chromatogram at 254 nm, 366 nm and 540 nm are depicted in the Figure I, II & III; Fig. IV, V & VI and Fig.VII, VIII & IX respectively.

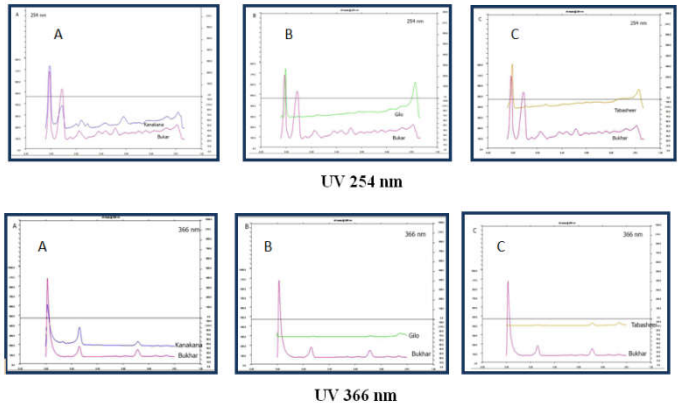


Figure 3 Comparative Densitometric chromatogram of Bukhar with Kanakana (A), Satt-e-Gilo (B) and Tabasheer (C) at UV 254 nm and 366 nm (Absorbance mode)

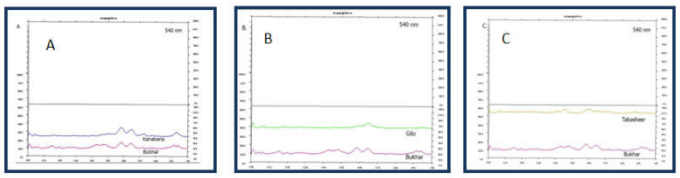


Figure 4 Comparative Densitometric chromatogram of Bukhar with Kanakana (A), Satt-e-Gilo (B) and Tabasheer (C) at 540 nm (Absorbance mode)

Antimicrobial activity studies

The results of antimicrobial activity studies of Habb-e-Bukhar against few medically important organisms were depicted in

the Figure 5. The drug has potent activity against all the organisms tested, irrespective of gram positive or gram negative cell walls. The drug has good activity even against the yeast culture *Candida albicans*. The broad-spectrum antibiotic ampicillin (10 mcg) was used as standard. The drug volume was taken as 75µl per well at the increasing concentration ranging from of 1.8µg, 18µg, 180µg and 1800µg. DMSO (75 µl), used as vehicle control, did not show any zone of inhibition, which clearly states that the developed zone of inhibition is due to the phyto-constituents of the drug. For most of the organism the minimum inhibitory concentration of the drug was found to be 18µg where the zone was seen clearly. In case of *S. aureus*, the standard ampicillin 10 mcg did not show any inhibition, whereas our test drug Bukhar proved to show inhibition. MIC was also found to be less than 1.8µg (Figure 5).

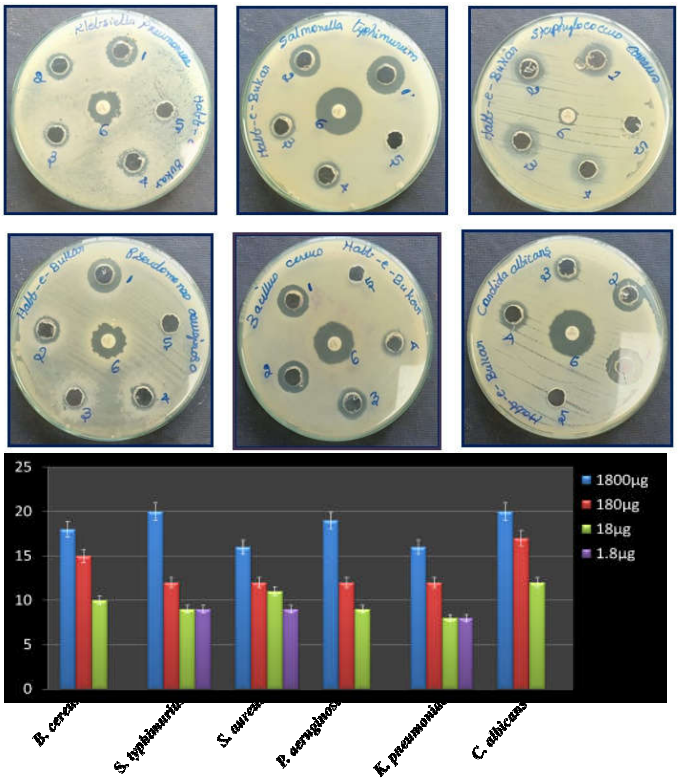
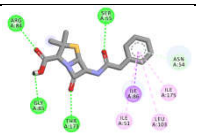
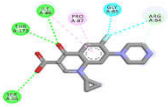
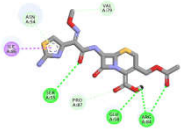
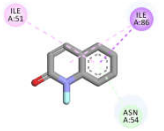
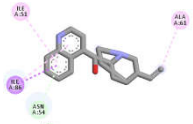
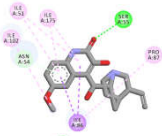
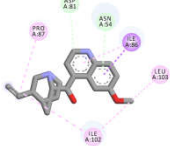


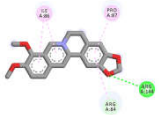
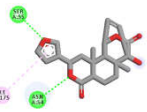
Fig 5 Antimicrobial activity studies of Habb-e-Bukhar against Few Microbial strains and its Minimum Inhibitory Concentration (MIC)

In-silico Activity studies:

In order to have a better understanding about the inhibitory mechanism as well as the mode of interactions of the phyto-constituents of the formulation docking analysis was performed. Two target proteins, DNA Gyrase and Penicillin binding protein of the gram positive organism, *S. aureus* were selected to analyse the mechanism of interaction for the study. The enzyme DNA Gyrase maintains the structure of DNA. It plays a crucial role in unwinding the DNA replication fork at the time of replication. Any inhibitors binding to the DNA Gyrase modifies the pathway of bacterial replication thereby arresting the growth and becomes bactericidal. In the present study all the tested phyto-constituents of Kanakana and Satt-e-Gilo have shown good docking results ranging from -7.1 to -8.4 kcal/mol (Table 2). The phyto-constituent of Satt-e-Gilo viz Berberine, Tinosporin have shown good docking results and had both hydrogen and hydrophobic interactions with the target amino acid residues of the enzyme *S. aureus* DNA Gyrase. The cinchona components also had better docking affinities ranging from -7.7 to -7.1 compared to the standard drugs Cefotaxime and Fluroquinolones. The other target site, PBP's are the proteins commonly found in many bacteria and are involved in the synthesis of final stage of peptidoglycan, the major component of bacterial cell wall. Cell wall synthesis is essential to growth, cell division and maintenance of cellular structure of bacteria. Any inhibitor that binds or blocks the PBP'S's leads to defects in cell wall structure and eventually becomes fatal. In our present study, both the tested phyto-constituents of Satt-e-Gilo and the Kanakana showed a very effective docking affinities ranging from -8.6 to -7.1 Kcal/mol (Table 3**)**. Indeed, Berberine and Tinosporin are comparatively effective than the selected control drugs. On the other hand, Tabasheer phyto-constituents, did not show any binding interactions with any of the targets proteins of *S. aureus*. It is assumed that Tabasheer could serve as nano carrier, since in the present days, interest on silica nanoparticles as a system to deliver drugs is increasing.

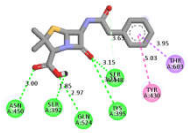
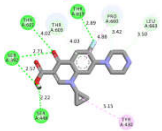
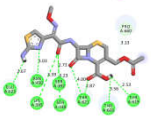
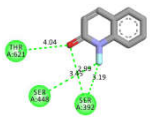
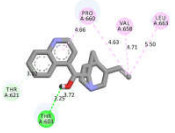
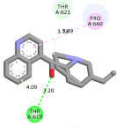
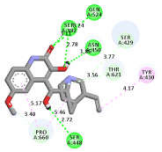
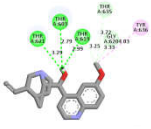
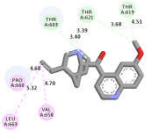
Table 2 Amino acid residues of DNA Gyrase (3G7B) with ligands

Compound	Binding Energy (Kcal/mol)	Interactions		2D Structure
		H-Bonding	Hydrophobic	
Penicillin G	**-8.8**	Ser55, Arg84, Gly85, Thr173	Ile51, Asn54, Ile86, leu103, Ile175	
Ciprofloxacin	**-7.9**	Ser55, Gly85, Ile86, Thr173	Arg84, Pro87	
Cefotaxime	**-6.7**	Ser55, Glu58, Arg84	Asn54, Val79, Ile86, Pro87	
Fluroquinolones	**-6.3**	NHB	Ile51, Asn54, Ile86	
Phytoconstituents of Kanakana				
Cinchonidine	-7.1	NHB	Ile51, Asn54, Ala61, Ile86	
Cinchonine	-7.6	Asn54	Ser55, Asp81, Ile86, Pro87, Ile102	
Dihydroxyquinidine	-7.4	Ser55	Ile51, Asn54, Ile86, Pro87, Ile102, Ile175	
Quinidine	-7.7	NHB	Asn54, Asp81, Ile86, Pro87, Ile102, Leu103	

Quinine	-7.7	NHB	Ile51, Asn54, Ala61, Ile86, Leu103	
Phytoconstituents of Satt-e-Gilo				
Berberine	-8.3	Arg144	Arg84, Ile86, Pro87	
Tinosporin	-7.8	Asn54, Ser55	Ile175	

NHB: No Hydrogen Bond Interactions; The ligand is shown in sticks and the interacting amino acid residues with their numbers are shown inside circles.

Table 3 Amino acid residues of Penicillin binding protein (3VSL) with ligands

Compound	Binding Energy (Kcal/mol)	Interactions		2D Structure
		H-Bonding	Hydrophobic	
Penicillin G	**-7.8**	Ser392, Lys395, Ser448, Asn450, Gln524	Tyr430, Thr603	
Ciprofloxacin	**-7.7**	Ser392, Ser448, Thr619, Thr621	Tyr430, Thr603, Pro660, Leu663	
Cefotaxime	-7.7	Ser392, Lys395, Ser448, Asn450, Thr603, Thr619, Thr621, Glu623	Pro660	
Fluroquinolones	-5.7	Ser392, Ser448, Thr621	-	
Phytoconstituents of Kanakana				
Cinchonidine	-7.2	Thr603	Thr621, Pro660, Val658, Leu663	
Cinchonine	-7.1	Thr619	Thr621, Pro660	
Dihydroxyquinidine	-7.6	Ser392, Ser448, Asn450, Gln524	Ser429, Tyr430, Thr621, Pro660	
Quinidine	-7.6	Thr603, Thr619, Thr621	Thr635, Gly620, Tyr636	
Quinine	-7.2	NHB	Thr603, Thr619, Thr621, Val658, Pro660, Leu663	
Phytoconstituents of Satt-e-Gilo				
Berberine	-8.6	NHB	Asn450, Val606, Tyr636, Pro660	
Tinosporin	-7.6	NHB	Thr619, Thr621	

NHB: No Hydrogen Bond Interactions.; The ligand is shown in sticks and the interacting amino acid residues with their numbers are shown inside circles.

CONCLUSION

The study has revealed that the drug Habb-e-Bukhar has a good antimicrobial activity against all the tested organisms. The phyto-constituents of Kanakana (*C. officinalis*) and Satt-e-Gilo (*T. cordifolia*) elucidated good hydrogen and hydrophobic interactions with the amino acid residues of two target sites DNA Gyrase and PBP's of *Staphylococcus aureus*. The role of Tabasheer in the formulation could be for sustained delivery of the drug component due to the presence of silica in it. Hence from the study data, it is perceived that the drug as a whole may yield a synergistic effect in controlling the organism suspected for infection and act as an effective biocompatible anti-therapeutic agent.

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References

1. Weeks J. Call to Action: Announcing the Traditional, Complementary and Integrative Health and Medicine Covid-19 support Registry. *Journal of Alternate and Complement Medicine* 2020; 26: 256-258. doi: 10.1089/acm.2020.29083.jjw, PMID 32250655.
2. NFUM. National formulary of Unani medicine. Part I, 1st Reprint. New Delhi: Ministry of Health and Family Welfare (Department of AYUSH), Government of India, 2006; p. 13.
3. Acremont VD, Kilowoko M, Esther K, Philipina S, Sangu W, Maro JK, *et al*. Beyond Malaria - Causes of Fever in Outpatient Tanzanian Children. *New England Journal of Medicine*; 2014; 27, 809-17.
4. Santajit S, Indrawattana N. Mechanism of Antimicrobial Resistance in ESKAPE Pathogens. BioMed Research International 2016; 5: 1-7. doi: 10.1155/2016/2475067, PMCID: PMC4871955.
5. Uttpal A, Herrera NJ, Altemimi A, Lakhssassi N. A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. Metabolites 2019; 9: 258. doi: 10.3390/metabo9110258.
6. Kacprzak KM, Chemistry and Biology of Cinchona Alkaloids. Natural products. Berlin, Heidelberg: Springer-Verlag; 2013. p. 605-41. doi: 10.1007/978-3-642-22144-6-22.
7. Gachelin G, Garner P, Ferroni E, Tröhler U, Chalmers I. Evaluating Cinchona Bark and quinine for treating and

preventing malaria. *Journal of the Royal Society of Medicine*. 2017; 110: 31-40. doi: 10.1177/01410768 16681421, PMID 28106483.
8. Sharma P, Bharat P, Dwivedee, Bisht D, Dash A K, Kumar D. The Chemical Constituents and Diverse Pharmacological Importance of Tinospora cordifolia. Heliyon 2019; 5: 1-8. doi: 10.1016/j.heliyon.2019.e02437, PMID: 31701036.
9. Tiwari P, Puravi N, Prusty SK, Sahu PK. Phyto-chemistry and Pharmacology of Tinospora cordifolia: a Review. Systematic Reviews in Pharmacy 2018; 9(1):70-8. doi: 10.5530/srp.2018.1.14.
10. Ahmad N, Nawab M. Qamar U. Medicinal Properties of Gilo (*Tinospora cordifolia*) - a Review. World Journal of Pharmacy and Pharmaceutical Sciences, 2019; 8: 430-40. doi:10.20959/wjpps20198-14332.
11. Sharma R, Amin H, Galib, Prajapati P. Validation of standard manufacturing procedure of Guḍuci sattva (aqueous extract of Tinospora cordifolia (Willd.) Miers) and its tablets. Ancient Science of Life, 2013; 33: 27-34. doi: 10.4103/0257-7941.134564, PMID: 25161327.
12. Pradhan D, Ashok KP. Estimation of starch extracted from different diameter thickness stem of Giloe (*Tinospora cordifolia* (Willd.) Miers.,). *Journal of Pharmacognosy and Phytochemistry*, 2013; 2: 162-4.
13. Maji JK, Patel M, Mehta PJ. Bambusha; Realm of Indian Traditional Medicine. *Nirma University Journal of Pharmaceutical Sciences*, 2018; 5: 65-72.
14. Aisha AA, Tabassum K, Ambar S. Tabasheer (Bambusa arundinacea Retz.) a plant origin drug of Unani Medicine - a review. *Journal of Ayurvedic and Herbal Medicine* 2019; 5: 31-34. doi: 10.31254/jahm. 2019.5109.
15. Kalluri J, Rodriguez RG, Hartman PS, Loni A, Canham L, Coffer J. Single plant Derived Nanotechnology for Synergistic Antibacterial Therapies. PLOS ONE 2016; 11:1-12. doi:10.1371/journal.pone.0163270.
16. European Commission. EU guidelines to Good manufacturing Practice. Medicinal Products for Human and veterinary use, Annex 7: Manufacture of Herbal Medicinal Products, 2008; 4:1-6. https://ec.europa.eu/ health/sites/health/files/files/eudralex/vol-4/vol4_an7_2008_09_en.pdf.
17. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd ed. Springer Berlin Heidelberg, Germany, 1996.
18. Collee JG, Mackie TJ, McCartney JE. Practical Medical Microbiology. Churchill Livingstone Publishers, New York, 1996.
19. IP, Pharmacopoeia of India. Microbiology Assays and Tests. Government of India, 1996.
20. Emran TH, Rahman MA, Nasiruddin MM, Dash R, Hossen MF, Mohiuddin M, *et al*. Molecular Docking and Inhibition Studies on the Interactions of Bacopa monnieri's potent phytochemicals against Pathogenic Staphylococcus aureus. DARU *Journal of Pharmaceutical Sciences* 2015; 23: 26. doi: 10.1186/s40199-015-0106-9, PMID: 25884228.
21. Gurung P, Puspal D. Spectrum of Biological Properties of Cinchona alkaloids: A Brief Review. *Journal of Pharmacognosy and Phytochemistry*, 2017; 6:162-166.
22. Klinowski J, Cheng CF, Sanz JS, Rojo J M, Mackay A. Structural Studies of Tabasheer, an opal of plant origin. *Philosophical Magazine Journal*, 1998; 77: 201-216. doi: 10.1080/014186198254704.

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