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RESEARCH ARTICLE

Studies on anti-microbial activity of *Quisqualis indica* L. plant extracts on urinary tract infection causing pathogens

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Abstract

The ornamental plant, *Quisqualis indica* L. has several pharmacognistic properties. The anti-bacterial activity of the plant extracts against common bacteria causing Urinary tract infections (UTIs) were determined. In this study, *Quisqualis Indica* L. plant samples were collected during the spring season from Kalahandi District of Odisha. The different solvent extracts of the medicinal plant, *Q. indica* were used for determining the anti-bacterial activity against human pathogenic micro-organisms isolated from samples of patients having UTIs. In all, urine samples from 110 patients having UTIs were analyzed. Antibiotic susceptibility of gram-positive and gram-negative bacterial isolates was studied. The Molsoft tool was used to identify the drug likeness score of each compound. *Escherichia coli* was observed in 57 %, *Pseudomonas aeruginosa* in 17 %, *Staphylococcus aureus* in 15 % and *Klebsiella pneumoniae* in 10 % of the samples. *Escherichia coli* was the most predominant bacteria isolated from UTIs. Seven bacterial strains were used in the study namely *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* and *Acinetobacter baumannii*. The extracts of *Q. indica* have significant anti-bacterial properties against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii* and *Streptococcus pyogenes* causing the UTIs. The compounds mainly Phytol, Trans-sequalene, Tocoferol, Vitamin-E -acetate and stigmasterol have higher drug likeness scores. Our study has reported the structural configuration of the extracts having higher drug-likeness score with a potential for future therapeutic drug against the UTIs. This study focusses on UTIs and the phytochemical analysis has been evaluated with drug likeness score.

Keywords: anti-microbial activity; pathogenic micro-organisms; plant extracts; *Quisqualis indica* L.

Introduction

Due to the unpropitious effects of synthetic drugs and the efficacy of traditional medicine in treating chronic diseases with fewer side effects, researchers have focused on the plant descended products. In the last two decades, the consumption of herbal medicine has increased consistently throughout the world as an auxiliary treatment for several health problems like diabetes, Urinary tract infections (UTIs) and other persistent illnesses. The abundance of secondary metabolites in plants combined with low cost of extraction has laid the foundation for extensive research into plant derived antimicrobials.

Urinary tract infection is one of the most prevalent forms of extra-intestinal infection that affects any part of the urinary tract attributable to pathogenic Gram-positive and Gram-negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pyogenes, Staphylococcus aureus and Acinetobacter baumannii.* The predominant etiological agent for both uncomplicated and complicated UTIs is *E. coli* followed by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (1, 2). UTIs are quite common due to several reasons including using unhygienic public toilets and not using sufficient water for cleaning the surfaces. UTIs happen much more frequently in women than in men, the ratio being 8 :1. UTIs also tend to recur (3). Antibiotics are the most common treatment for UTIs. Increasingly, researchers have been exploring to determine whether non-antibiotic treatments can resolve UTIs. In spite of the availability of potent antibiotics, resistant and multi-drug resistance isolates are assiduously emerging. Several medicinal plants have been screened for potential anti-bacterial activity due to the increasing failure of antibiotics, related toxicity issues and rise of resistant pathogens (4-6). *Quisqualis indica L.,* a common, popular, ornamental garden climber grown shows a wide range of remarkable medicinal properties. The pharmacognistic properties of *Q. indica* have been reviewed by previous researchers (7). In this study, the activity of crude extracts of the medicinal plant, *Q. indica* was investigated against human pathogenic micro-organisms isolated from samples of patients having UTIs. Antibiotic susceptibility of the Gram-positive and Gram-negative strains of bacteria was studied. Selective compounds were screened for the minimum inhibitory concentration. In order to identify the drug likeness score of each compound as per their structure canonical 'simplified molecular-input line entry system', Molsoft tool was

used. Drug-likeness is an important criterion used to assess the potential of compounds for drug development. This indicator helps to screen out compounds that may fail in clinical trials at an early stage, which is important for increasing the success rate of drug development and reducing costs (8-10). Lipinski's Rule of 5 is a guideline in drug discovery that predicts oral drug absorption and permeability, stating that a compound is likely to have good oral bioavailability if it has no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, a molecular mass under 500 Daltons and an octanol-water partition coefficient (LogP) not greater than 5. Extracts were subjected to molecular properties prediction, drug-likeness by Molinspiration (Molinspiration, 2008) & MolSoft (MolSoft, 2007) software.

Materials and Methods

Collections of plant samples

Quisqualis indica L. plant samples were collected during the spring season (March, 2018, The plant's flowering stage) from Kalahandi District of Odisha, India (19.9137° N, 83.1649° E) in the South-Western parts of the State which is known for its rich bio-diversity conservation and ethnobotany (11, 12).

Preparation of crude extracts of the plants

The aerial parts of *Quisqualis indica* L. were washed thoroughly to remove dust and left in a dark corner of the laboratory at room temperature for 10-15 days for drying, avoiding direct sun light. The stem, leaves and flowers were ground to a fine powder by mortar and pestle and then stored in a tight closed container until further use.

Extraction was carried out as per the methods described by previous researchers, ground samples of *Quisqualis indica* L. aerial parts (Leaves and flowers) were measured and shifted to a container for extraction using Soxhlet apparatus. Ground samples were finely concentrated sequentially with solvents, namely Double distilled water, Methanol, Petroleum ether and n- Hexane, in order of increasing polarity, using 200-250 mL of each solvent at room temperature. The samples were centrifuged (3000 rpm, 5 min.) to eliminate the impurities and suspended solids and the supernatant were used as crude extracts. The filtered residues were dried by evaporation at 40 ºC in a water bath. Lastly, the dry extract was measured and concentration of each of the extracts was determined and stored at -20 ºC for determining the anti-bacterial activity (13, 14).

Collection of clinical samples

In all, urine samples from 110 patients having Urinary tract infections (UTIs) were collected from the Pathology department of Capital Hospital, Bhubaneswar. Of these, 73 were from females and 37 from males. Informed consent was obtained before collection of samples.

Microbial culture of samples & sub-culturing of bacterial strains

Seven pathogenic bacterial strains (4 cultured from clinical samples and 3 ATCC cultures) were used in the study namely *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* (ATCC 12384) and *Acinetobacter baumannii* (ATCC 19606). In order to identify the selected micro-organisms, various morphological, physiological and conventional biochemical tests were carried out sequentially. For culturing the clinical samples, Nutrient broth was utilized. Different culture media (Mac Conkey agar, Nutrient agar, Eosin methylene blue agar, Luria Agar and Mannitol salt agar) were prepared according to instructions of the manufacturer (HI-MEDIA, India). For sub-culturing of bacterial cultures, inoculation on nutrient broth was followed by incubation overnight at 37 ºC (15).

Anti-microbial susceptibility testing

Anti-microbial susceptibility testing of all identified isolates was done according to the criteria of the Clinical and Laboratory Standards (16). The methods described by Sood, 2012 & Choudhury, 2015 were followed (17, 18).

Anti-microbial assay of plant extracts and determination of zone of inhibition

The elaborate methods described by Saha, 2014 and Wayne, 2011 were followed for anti-microbial assay of plant extracts and determination of the zone of inhibition (19, 20).

Evaluation of minimum inhibitory and bacterial concentrations

The methods described by Mishra, 2013 & Prakash, 2013 were followed for the determination of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) (21-23).

The MIC and MBC of the 4 active plant extracts, prepared with Aqueous, Methanol, Petroleum ether and Hexane were determined by suitable dilution from original stock solution of aerial part extracts for concentration of 0 mg/mL, 45 mg/mL, 22.5 mg/mL, 11.25 mg/mL 5.62 mg/mL, 2.81 mg/mL, 1.40 mg/mL, 0.70 mg/mL and 0.35 mg/mL in aliquots of 10 % DMSO solution. For each of the extracts, all the experiments were conducted in triplicates.

Gas Chromatography-Mass Spectroscopy analysis (GC-MS)

GC-MS analysis of *Quisqualis indica* L. Methanol, Petroleum ether and n -hexane leaf extract was carried out on a 7000 D Triple Quadrupole GC/MS with a column TG 5MS (30 m × 0.25 mm, 0.25 µm). Helium was used as a carrier gas at a flow rate of 1 mL/min. Split / Splitless (S/SL) injector was used with 250 ºC injector temperature. 1.0 µL sample injection volume was utilized. Ion source temperature was maintained at 230 ºC. The oven temperature was programmed initially at 80 ºC for 2 min, then programmed to increase to 280 ºC at a rate of 5 ºC/min ending with a 5 min isothermal at 280 ºC. Total run time was 38.10 min, 34.22 min and 36.08 for methanol, petroleum ether and n-hexane, respectively. The MS transfer line was maintained at a temperature of 250 ° C. 7000D Triple Quadrupole MS detector was used for analysis and data was evaluated using mass hunter for compound identification and quantification. The NIST 23 Mass Spectral Library & Search Software (NIST 2023/2020/2017/EPA/NIH) were used.

Qualitative phytochemical analyses

Different qualitative phytochemical analyses were carried out for *Q. indica* crude extracts. Anti-microbial activity could be attributed to the secondary metabolites namely alkaloids, flavonoids, carbohydrates, terpenoids, steroids, tannins, saponins, etc. present in the plant extracts. From pubChem database, structure and related information of anti-microbial compounds were analyzed. The Molsoft tool L.L.C., 2021 was used to find out the drug likeness score of each compound as per the structure canonical 'simplified molecular-input line entry system'.

Statistical analysis

Descriptive statistics were used for evaluating the baseline characteristics of plant extracts. Quantitative variables were expressed in mean ± SD. The results were analyzed using ANOVA and t - tests for comparing the groups. The performances of diagnostic test were expressed in terms of MIC and MBC. SPSS version 25 was used for statistical analysis.

Results and Discussion

In all, urine samples from 110 patients having UTIs were collected from pathology department of Capital Hospital, Bhubaneswar. Of these, 73 (66%) were from females and 37 (33 %) from males. Table 1 shows the frequency of microbes isolated from the urine samples of patients with UTIs. *Escherichia coli* was observed in 57 % (63), *Pseudomonas aeruginosa* in 17 % (19), *Staphylococcus aureus* in 15 % (17) and *Klebsiella pneumoniae* in 10 % (11) of the samples. *Escherichia coli* was the predominant bacteria isolated from UTIs. More females (15) were having *Pseudomonas aeruginosa* whereas more males (8) were harboring *Staphylococcus aureus* bacterial infections. The antibiotic susceptibility of Gram-positive and Gram-negative bacterial isolates is depicted in Table 2. High sensitivity was observed by *Escherichia coli* strains to Gentamycin, Ceftazidime, Cephalexin, Amoxicillin, Vancomycin followed by Ciprofloxacin, Azithromycin and Erythromycin. This strain exhibited resistance against Ofloxacin and Amikacin. *Staphylococcus aureus* strains were sensitive to Erythromycin, Azithromycin, Amikacin, Ofloxacin followed by Ciprofloxacin, Tetracyclin and resistant to Gentamycin, Amoxicillin and Vancomycin. *Klebsiella pneumoniae* strains were sensitive to only 4 drugs namely Ceftriaxone, Ceftazidime, Piperacillin and Amikacin. *Pseudomonas aeruginosa* demonstrated sensitivity to 6 drugs (Gentamycin, Ceftazidime, Cephalexin, Amoxicillin, Ofloxacin and Amikacin) and resistance to 2 drugs (Ceftriaxone and Ciprofloxacin). *Acinetobacter baumannii* (ATCC 19606) showed sensitivity to only Amikacin, resistance to 2 drugs and no activity towards other drugs. *Streptococcus pyogenes* demonstrated sensitivity to only 3 drugs namely Ofloxacin, Azithromycin and Erythromycin. Further, it showed either indeterminate or no activity to the other drugs. After checking the antibiotic susceptibility of the bacteria, the plant extracts were tested for zone of inhibition followed by MIC/MBC of the most active extracts and then the GC-MS analysis was carried out. Selected compounds were screened for the minimum inhibitory concentration. The anti-microbial activity as demonstrated by the size of zone of inhibition of *Quisqualis indica* L. plant extracts against tested bacterial isolates with gentamycin 30 µg/mL as the positive control is depicted in Table 3. The Aqueous and Ethyl acetate extract of *Quisqualis indica* L. exhibited the maximum value of inhibition zone of 13 mm, against *Escherichia coli* and the minimum value of 9 mm and 7 mm for *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (ATCC 19606). The n-Hexane extract demonstrated inhibition against all the bacterial isolates, *Escherichia Coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii* (ATCC 19606) and *Streptococcus pyogenes* (ATCC 12384). Petroleum ether extract showed inhibition against *Escherichia Coli, Staphylococcus aureus* and *Streptococcus pyogenes*. Methanol extracts could inhibit only 3 bacterial isolates namely *Staphylococcus aureus, Acinetobacter baumanni* and *Streptococcus pyogenes* (ATCC 12384). Table 4 shows that the aqueous extract of *Q. indica* had the minimum MIC value, 2.81 mg/mL and the minimum MBC value 5.62 mg/mL against *Escherichia coli* and *Staphylococcus aureus,* while a maximum MIC and MBC value against *Pseudomonas*

Table 1. Shows the percentage of microbes isolated from the urine samples

Microorganisms	Females n (%)	Males n (%)	Frequency n (%)	Mean ± SD (%)
Escherichia coli	42 (57.53)	21 (56.75)	63 (57.27)	57.18 ± 0.39
Klebsiella pneumoniae	7 (9.58)	4 (10.81)	11 (10)	10.13 ± 0.63
Pseudomonas aeruginosa	15 (20.54)	4 (10.81)	19 (17.27)	16.21 ± 4.95
Staphylococcus aureus	9 (12.32)	8 (21.62)	17 (15.45)	16.46 ± 4.73
Total	73	37	110	24.83 ± 18.95

Table 2. Depicts the responses of Gram-positive bacteria to different antimicrobial agents

Antibiotics	*Staphylococcus aureus*		Mean ± SD
AM - Ampicillin	S = 12	R = 5	8.5 ± 4.95
CIP - Ciprofloxacin	S = 15	R = 2	8.5 ± 6.50
CM - Clindamycin	S = 16	R = 1	8.5 ± 7.50
CRO - Ceftriaxone	S = 10	R = 7	8.5 ± 2.12
ERY - Erythromycin	S = 11	R = 6	8.5 ± 3.54
GM - Gentamicin	S = 15	R = 2	8.5 ± 6.50
TE - Tetracycline	S = 13	R = 4	8.5 ± 4.50
VA - Vancomycin	S = 15	R = 2	8.5 ± 6.50

Table 3. Depicts the responses of Gram-negative bacteria to different antimicrobial agents

Antibiotics	*Escherichia coli*		*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*	
AM - Ampicillin	S = 44	R = 19	S = 2	R = 9	S = 0	R = 19
AMC - Amoxicillin	S = 40	R = 23	S = 7	R = 4	S = 2	R = 17
AN - Amikacin	S = 52	R = 11	S = 16	R = 2	S = 1	R = 18
CAZ - Ceftazidime	S = 57	R = 6	S = 10	R = 1	S = 15	R = 4
CIP - Ciprofloxacin	S = 54	R = 9	S = 10	R = 1	S = 10	R = 9
CRO - Ceftriaxone	S = 49	R = 14	S = 8	R = 4	S = 8	R = 11
GM - Gentamicin	S = 59	R = 4	S = 9	R = 2	S = 2	R = 17
PIP - Piperacillin	S = 10	R = 53	S = 1	R = 10	S = 1	R = 8
Mean ± SD	**32.06 ± 20.55**		**5.94 ± 4.12**		**8.81 ± 6.38**	

Table 4. Shows the antimicrobial activity as size of zone of inhibition of *Quisqualis indica* L. plant extracts against tested bacterial isolates with gentamycin 30 µg/mL as the positive control

Bacteria	Size of zone of inhibition by different solvent extracts (mm) of *Quisqualis indica L*			
	Methanol	Petroleum ether	n-Hexane	Gentamycin 30 µg/ml
Escherichia coli	13	13	17	22
Staphylococcus aureus	14	15	14	25
Pseudomonas aeruginosa	13	17	17	23
Klebsiella pneumoniae	17	13	14	27
***Acinetobacter baumannii* (ATCC - 19606)**	12	18	17	25
***Streptococcus pyogenes* (ATCC- 12384)**	16	14	19	27
Mean ± SD	**14.17 ± 1.94**	15.00 **±** 2.16	16.33 **± 1.97**	24.83 **±** 1.94

aeruginosa, *Klebsiella pneumoniae*, *Acinetobacter baumannii* (ATCC 19606) and *Streptococcus pyogenes* (ATCC 12384). Table 5 shows the Minimum inhibitory concentration (MIC) and Minimum Bactericidal concentration (MBC) of *Q. indica* plant extracts against tested bacterial isolates. The methanol extract of *Q. indica* showed the lowest MIC value of 5.62 mg/mL and the lowest MBC value of 11.25 mg/mL against *Streptococcus pyogenes* (ATCC 12384) and no growth for *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* detected at the tested concentrations. Whereas the Petroleum ether extract showed MIC value of 2.81 mg/mL and MBC value of 5.62 mg/mL against *Staphylococcus aureus* and *Acinetobacter baumannii* (ATCC 19606), MIC value of 5.62 mg/mL and MBC value of 11.25 mg/mL against *Escherichia coli* while no growth was observed for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at the tested concentrations. Ethyl acetate extract showed lowest MBC value of 2.81 mg/mL and MIC value of 5.62 mg/mL against *Pseudomonas aeruginosa* only. The hexane extract showed the lowest MIC value of 5.62 mg/mL and the lowest MBC value of 11.25 mg/mL against *Escherichia coli, Pseudomonas aeruginosa* and *Acinetobacter baumannii* (ATCC 19606).

Statistical analysis: ANOVA for MIC and MBC

Significant difference ($p = 0.042$) in MIC values among bacterial strains was observed. *Klebsiella pneumoniae* showed the highest MIC, while *Escherichia coli* and *Pseudomonas aeruginosa* had the lowest. Significant variation ($p = 0.037$) was found in MBC values among bacterial strains. *Klebsiella pneumoniae* again had the highest MBC, indicating lower sensitivity to the extracts. A lesser MIC/MBC value denotes that a minimum amount of plant extracts is used, whereas a higher value denotes the use of comparatively more volume of plant extracts for the restriction of any bacterial species.

Table 5 shows the details of the compounds with Drug likeness score. The extracts showed drug likeness score of varying degrees ranging from 0.16 to 1.10 with Stigmasterol, Urosolic acid, Lupeol, Tocoferol, Vitamin E acetate, Trans Squalene, Methyl isohexadecanoate, Isobutyl-o-phthalate, Phytol and Cycloartenyl acetate.

GC-MS analysis

GC-MS chromatograms of methanol extract of *Q. indica* for different retention time are given. The number and nature of phytochemical constituents as depicted by the peaks were characterized and identified by comparing the mass spectra of the constituents with NIST library ensuring an acceptable match score for each compound. The NIST 23 Mass Spectral Library & Search Software (NIST 2023/2020/2017/EPA /NIH) were used. Phytochemical analysis of *Q. indica* by GC-MS analysis revealed five peaks corresponding to the presence of different compounds. The analysis identified several bioactive compounds, including Stigmasterol, Urosolic acid, Lupeol, Tocoferol, Vitamin E acetate, Trans-squalene, Methyl iso-hexadecanoate, Isobutyl-o-phthalate, Phytol and Cycloartenyl acetate. Fig. 1 shows the molecular interactions of two most potential phyto-constituent docking complexes. The molecular interaction was presented by BIOVIA-Discovery Studio Vizualizer-2021.

In this study, the crude plant extracts of *Quisqualis indica* L. were used for determining the anti-microbial activity of *Q. indica* showed different levels of anti-microbial activity against both antibiotic sensitive and resistant bacteria namely *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii* (ATCC 19606) and *Streptococcus pyogenes* (ATCC 12384). A total of 110 urine samples from patients (73 females and 37 males) having UTIs were collected. Out of these, 74 % of the bacterial isolates were Gram- negative and 26 % were Gram-positive. *Escherichia coli* was the most common bacteria causing UTIs. *Escherichia coli* strains showed higher sensitivity to Gentamycin, Ceftazidime, Cephalexin, Amoxicilin, Vancomycin followed by Ciprofloxacin, Azithromycin and Erythromycin. This strain exhibited resistance against Ofloxacin and Amikacin. *Staphylococcus aureus* strains were sensitive to Erythromycin, Azithromycin, Amikacin, Ofloxacin followed by Ciprofloxacin, Tetracycline and resistant to Gentamycin, Amoxicilln and Vancomycin. Aqueous, Petroleum ether, Ethyl acetate and Hexane extract of the plant *Quisqualis indica* L. showed good anti-microbial activity against most of the sensitive and resistant isolates, whereas Methanol extract had minimum activity against all the isolates. Aqueous and Ethyl acetate extracts of the plant exhibited

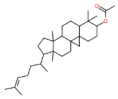
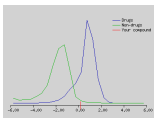
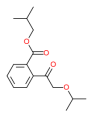
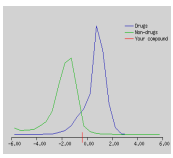
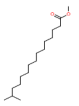
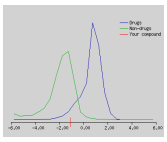
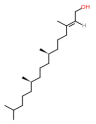
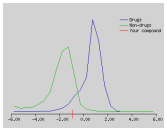
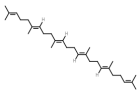
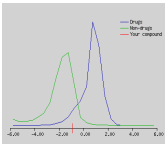
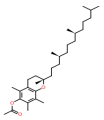
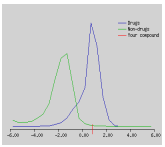
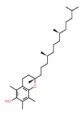
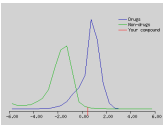
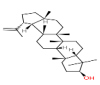
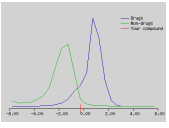
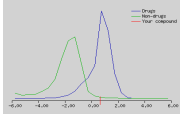
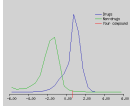
A. One-Way ANOVA for MIC Values

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-value / p-value
Between Groups	84.3	5	16.86	F = 2.73, p = 0.042
Within Groups	148.3	24	6.18	-
Total	232.6	29	-	-

B. One-Way ANOVA for MBC Values

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-value / p-value
Between Groups	190.8	5	38.16	F = 2.86, p = 0.037
Within Groups	320.1	24	13.34	-
Total	510.9	29	-	-

Table 5. Shows the details of the compounds with drug likeness score

Compound with their IUPAC name and chemical structure	Details of the composition	Drug-Likeness score
Cycloartenyl acetate 	**Molecular formula: C32 H52 O2** **Molecular weight: 468.40** **Number of HBA: 2** **Number of HBD: 0**	**Drug-likeness model score: 0.17** 
B. Isobutyl-o-phthalate 	**Molecular formula:** C16 H22 O4 **Molecular weight:** 278.15 **Number of HBA:** 4 **Number of HBD:** 0	**Drug-likeness model score:** -0.38 
C. Methyl isohexadecanoate 	**Molecular formula:** C17 H34 O2 **Molecular weight:** 270.26 **Number of HBA:** 2 **Number of HBD:** 0	**Drug-likeness model score:** -1.10 
D. Phytol 	**Molecular formula: C20 H40 O** **Molecular weight: 296.31** **Number of HBA: 1** **Number of HBD: 1**	**Drug-likeness model score: -0.87** 
E. Trans Squalene 	**Molecular formula: C30 H50** **Molecular weight: 410.39** **Number of HBA: 0** **Number of HBD: 0**	**Drug-likeness model score: -0.90** 
F. Vitamin E acetate 	**Molecular formula:** C31 H52 O3 **Molecular weight:** 472.39 **Number of HBA:** 3 **Number of HBD:** 0	**Drug-likeness model score:** 0.86 
G. Tocoferol 	**Molecular formula: C29 H50 O2** **Molecular weight: 430.38** **Number of HBA: 2** **Number of HBD: 1**	**Drug-likeness model score: 0.48** 
H. Lupeol 	**Molecular formula: C30 H50 O** **Molecular weight: 426.39** **Number of HBA: 1** **Number of HBD: 1**	**Drug-likeness model score: -0.22** 
I. Urosolic acid 	**Molecular formula: C30 H48 O3** **Molecular weight: 456.36** **Number of HBA: 3** **Number of HBD: 2**	**Drug-likeness model score: 0.66** 
J. Stigmasterol 	**Molecular formula:** C29 H48 O **Molecular weight:** 412.37 **Number of HBA:** 1 **Number of HBD:** 1	**Drug-likeness model score:** 0.62 

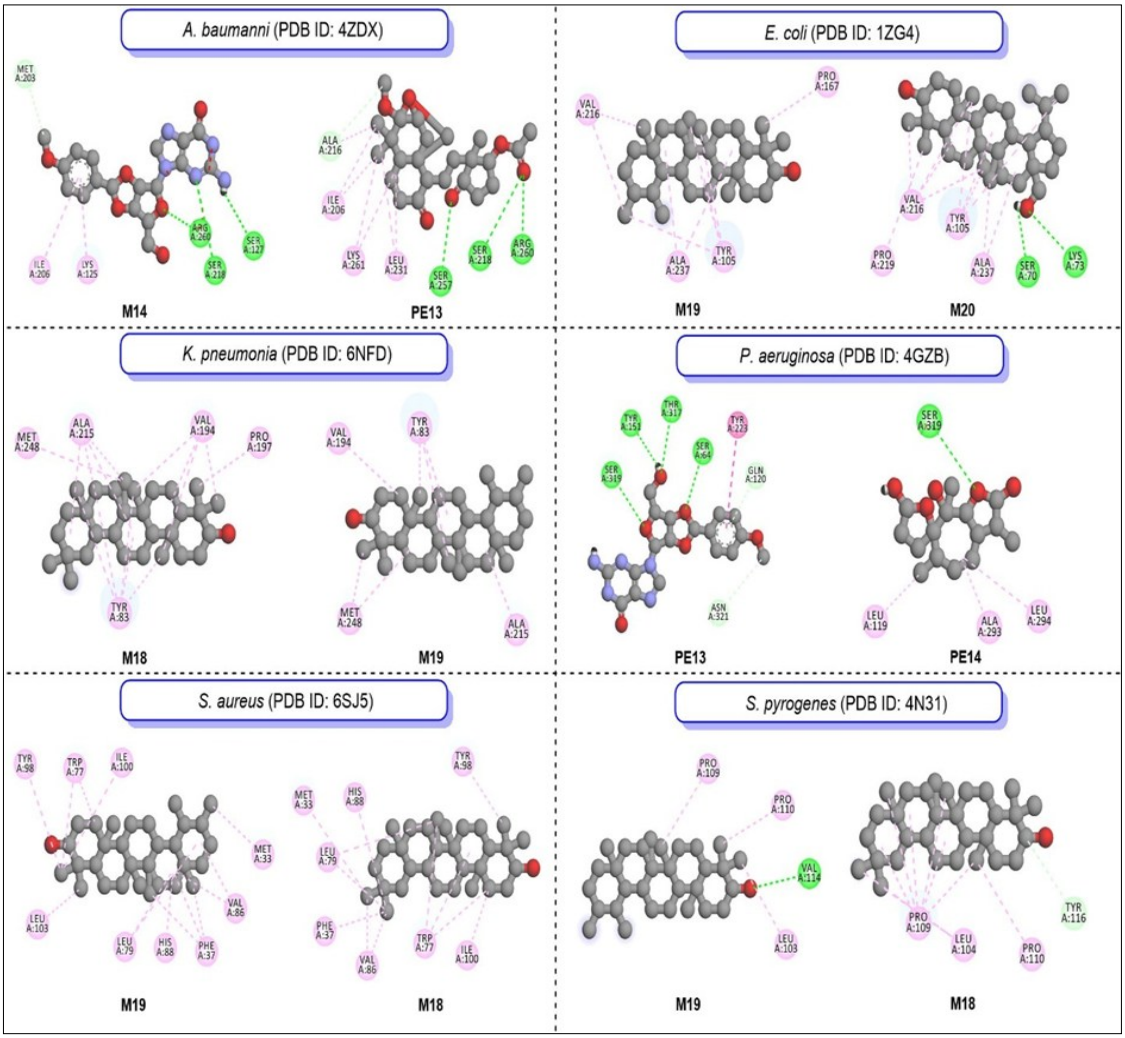


Fig. 1. Shows the molecular interactions of two most potential phyto-constituent docking complexes. The molecular interaction was presented by BIOVIA-Discovery Studio Vizualizer-2021.

Table 6. Shows the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Quisqualis indica* L. plant extracts against tested bacterial isolates

Bacteria	Different concentration of extracts against tested bacterial isolates with reference to MIC and MBC								
	Methanol		Petroleum Ether		n-Hexane		Gentamycin		Mean ± SD
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	(MIC/MBC)
Escherichia coli	5.62	11.25	5.62	11.25	5.62	11.25	1.87	3.75	4.68 ± 1.87 / 9.38 ± 3.70
Staphylococcus aureus	11.25	22.5	2.81	5.62	11.25	22.5	0.93	1.87	6.56 ± 5.81 / 13.62 ± 10.38
Pseudomonas aeruginosa	5.62	11.25	5.62	11.25	5.62	11.25	1.87	3.75	4.68 ± 1.87 / 9.38 ± 3.70
Klebsiella pneumoniae	11.25	22.5	11.25	22.5	11.25	22.5	3.75	7.5	9.38 ± 4.17 / 18.75 ± 7.22
Acenatobacter baumannii (ATCC- 19606)	11.25	22.5	2.81	5.62	5.62	11.25	7.5	15	6.80 ± 3.95 / 13.84 ± 7.67
Streptococcus pyogenes (ATCC- 12384)	5.62	11.25	11.25	22.5	-	-	7.5	15	8.12 ± 3.38 / 16.25 ± 5.78

large inhibition zones against the studied bacterial strains. Moreover, those people who prefer traditional means of treatment for various ailments use the aqueous extracts of plant parts *Q. indica*. This proves that meaningful anti-microbial activity against bacterial isolates is exhibited by the aqueous extracts of plants. Further, chemical composition analysis revealed that *Q. indica* extracts with anti-microbial activities contain alkaloids, flavonoids, tannins, sterol and other secondary metabolites. The common constituents found in all the four extracts (Aqueous, Methanol, Ethyl acetate and hexane) were Phytol, Trans-sequalene, Tocoferol, Vitamin-E-acetate and stigmasterol as shown in Table 6. Similar phytocompounds from *Quisqualis indica* L. in GCMS analysis were reported earlier (24-26). In previous reports, the antimicrobial activity of petroleum ether extract of *Quisqualis indica* flowers using agar well-diffusion method against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* showed best antimicrobial activity. MIC values were 27.0, 30.0, 38.0 and 40.0 µg/mL for *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilis,* respectively (24). A larger inhibition zone ~20-26 mm at 50 mg/mL for *Staphylococcus aureus* were reported earlier (24), whereas 13 mm was observed at similar concentrations in our study. This could be due to different plant parts, different bacterial strains and methodologies carried out after nearly one decade. In former studies, the active phytochemicals of 21 plants and suggested that screening of medicinal plants should be carried out to explore the specific therapeutic potential of anti-UTIs. Further, structural elucidation along with stereochemistry from isolated compounds of potent plants might be helpful for novel drug designing for

treatment of UTIs (27). There are many reports from different regions of the country on the anti-microbial activity of medicinal plants on the pattern of susceptibility of bacterial pathogens causing UTIs (28-30). Several authors have reported the medicinal properties, pharmacognistic, phytochemical and pharmacological aspects of *Q. indica* using different parts of the plant (31, 32). Our study has reported the structural configuration of the phytocompounds using different solvent extracts having higher drug-likeness score. These compounds could serve as leads and the extracts could potentially be developed into treatments.

Strengths and limitations

This study has specific strengths and some constraints. This is an *in vitro* study and we have used the different solvents extracts of the plants against some of the common bacterial strains from urine samples of patients with UTIs. However, we could not identify which specific compound is most active owing to the crude extracts. More resources and research are required for validation by means of *in vivo* experimentation.

Conclusion

We have observed that the different extracts of *Quisqualis indica* L. have meaningful anti-bacterial properties against some of the common UTI causing bacteria namely *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii* (ATCC 19606) and *Streptococcus pyogenes* (ATCC 12384). *Q. indica* extracts could serve as a source of new anti-microbial agents with the caveat of needing further in vivo study. The compounds namely Phytol, Trans-sequalene, Tocoferol, Vitamin-E-acetate and stigmasterol isolated from the extracts of the plant have higher drug likeness score. Hence, these extracts have the potential to be used for preparing drugs in future.

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Authors' contributions

BSB was involved in searching literature, conducted the experiments, analysed the data and has compiled the article. SSS was involved in bioinformatics analysis and final draft. RNP and PSK conceptualized the idea and designed the project proposal. TH supervised the study, reviewed and edited the article throughout all stages. SP facilitated the study by providing all necessary support. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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