Original Research

Antimicrobial Susceptibility of Plant Extracts on Isolated Microorganism from Diabetic and Non-Diabetic Population

Syeda Aatika Batool, Farheen Ansari, Sidra Gondal, Mahreen Mahmood, Kainat Asmat

ABSTRACT

Objectives: To isolate and characterizing microorganisms from diabetic and non-diabetic patients and assessing the antimicrobial activity of isolate extracts from Citrus sinensis and Psidium guajava.

Methodology: Experimental study of one year and six month duration from May 2016 to May 2018 conducted in Microbiology Laboratory University of Lahore, Pakistan. Convenient sampling was done by collecting the urine sample at random from 250 persons in the Pattoki community. All the urine samples from diabetic and non-diabetics were cultured separately and after biochemical confirmation of microorganisms, the disc diffusion method was used for carrying out an antimicrobial activity.

Results: A total of 105 samples, were found to have positive urine cultures. Among these positive urine culture samples, 75 were diabetic and 30 were non-diabetics. The bacterial isolate most commonly found among diabetics was E.coli followed by the presence of Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris respectively. Minimal Inhibitory Concentration (MIC) of different parts of plant extracts against isolated bacteria from diabetics showed that the highest sensitivity was shown against peel, leaves and seed extracts of Citrus sinensis and Psidium guajava while the least sensitivity was shown against the stem and root extracts of these plants. Moreover, antibiotic sensitivity tests of isolated microbes showed that the highest resistance is found against Augmentin and Tetracycline while the most sensitive drug for isolates was found to be Chloramphenicol.

Conclusion: From the results, it is concluded that leave extract of Psidium guajava and peel extracts of Citrus sinensis showed effective results against bacterial pathogens and could serve as a good alternate source of antibacterial agents.

KEYWORDS: Urinary Tract Infection, Asymptomatic Bacteriuria, Psidium guajava, Citrus sinensis, Diabetics

INTRODUCTION

Egyptians were the first who discovered diabetes and featured it through polyuria and weight loss. Greek physician Aertaeus designed the term Diabetes Mellitus (DM). Diabetes meaning is “to pass through’’ and Mellitus is the Latin word for “Honey’’ (sweetness) owing to the increased amount of sugar in the urine. It is surveyed and estimated that the prevalence of diabetes in adults has been increasing from 4% in 1995 to 6.4 % by 2025. The immune system of man is seriously disturbed and many abnormalities and complications are due to diabetes. It may lead to high saccharide concentration in the urine. Many types of disease-causing microbes can grow on this particular medium. The miserable infection condition and its risks may develop in diabetes patients at a greater rate.[1] Diabetics have a 10-fold increased risk of UTI as compared to non-diabetics and also a longer hospitalization.[2]

All over the world, urinary tract infections of acute nature occur in females and it becomes costly to take care of annually in Pakistan. It is estimated that 65% of all females experience one- or two-times urinary tract infections within their lifetime and 25 to 30% of females experience this infection repeatedly.[3] Early marriages are considered a factor for UTI in slim girls in Pakistan. Bacteria of

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variable pathogenicity like E. coli is most frequently responsible for urinary tract infections. Different other species like Klebsiella, Enterococcus, Proteus and Enterobacter spp. are also responsible for causing UTIs. Staphylococcus aureus, and Streptococcus group B are mostly observed for the enhanced ratio of UTI patients.[4]
Multidrug resistance of E. coli is due to geographic changes among other strains. E. coli is responsible for more than 82% of less complicated community-acquired UTIs, especially in females below 50 years of age. The prevalence of urinary tract infections (UTIs) and asymptomatic bacteriuria (ASB) increases in patients with diabetes mellitus (DM). People with diabetes are also at higher risk for complications of UTI and UTIs caused by fungi.[5]Diabetics are reported to have a three to four times higher risk of UTIs in comparison to non-diabetics. One reason suggested behind this increased bacterial growth is a dysfunctional bladder that promotes static pools of urine due to poor contraction. While another reason is bacterial growth and colonization promoted due to hyperglycemic urine.[6]
The synergistic effect of the extracts of plant parts containing many phytochemicals proved as best antimicrobial agents. These activities of the plant extracts (antimicrobial and other biological) depend upon the origins and extraction of plant parts. In traditional medicine, one of the most effective herbs is from the genus Citrus and further belongs to the family Rutaceae. In Asia, tropical and subtropical areas support the growth of the Citrus genus.[7]Major activities recognized in the Citrus species are antimicrobial, physiological, and pharmacological activities, medicinal, antioxidant, antinflammatory, hypoglycaemic and anticancer.[8] Rich sources of biologically active compounds are essential oils with antioxidant, antibacterial, insecticidal, antiviral and antifungal properties.[9] The citrus peel contains bioflavonoids (naringin and hesperidin) that result in the diabetic kineticism of orange.[10] Although many studies have been done on the antioxidant and antibacterial effect of juice and edible parts, there is meager literature on the wastes of citrus fruits of lemon and oranges of different varieties.[11] Plants in the family Myrtaceae have medicinal properties too such as guave or Psidium guajava, eucalyptus, allspice and clove. Guava is a native fruit of tropical America, but many tropical and subtropical countries cultivate it as edible fruit.[12] According to the literature, guava leaves also have an anti-diabetic effect so they could prove to be the best source of drug used for UTIs in diabetics.[13,14] Previously prevention and treatment of diarrhea were achieved by using guava leaves, roots and fruits. Guava leaves were detected to have a high level of antibacterial activity.[15] Significant antibacterial activity of guava reported in several studies against diarrhea-causing and food-borne bacteria such as E. coli, Salmonella & Shigella species, Staphylococcus species, Clostridium species, Bacillus species, and Pseudomonas species as food spoilage bacteria. Guava leaves and fruit juice tested in the treatment of many infections.[16]Psidium guajava leaves, fruit, bark, and roots proved by pharmacological investigations that they possess anti-inflammatory, antibacterial, analgesic, antipyretic, hypoglycemic, CNS depressant and spasmolytic activities.[17] The synergistic effect of the extracts of plant parts containing many phytochemicals proved as best antimicrobial agents[18]. There is a negative effect on microbial cells by plant-derived substances through various mechanisms of action as these substances attack the phospholipid bilayer of the cell membrane and destroy the enzyme system.[18] Moreover, there is an increased popularity in the application of natural antimicrobials due to a series of issues related to the control of microorganisms and as a source of pharmaceutical active compounds.[19,20] The objectives of this study were to isolate and characterize microorganisms from diabetic and non-diabetic patients and to check the antimicrobial susceptibility of isolatedmicroorganisms against C. sinensis and P. guajava.

METHODOLOGY

Experimental study of one year and six month duration from May 2016 to May 2018 conducted in Microbiology Laboratory University of Lahore, Pakistan. Convenient sampling was done by collecting the urine sample at random from 250 persons in the Pattoki community. All the urine samples from diabetic and non-diabetics were cultured separately. The first growth of microorganisms was on nutrient agar. The isolates were identified microscopically (Gram staining, shape) and macroscopically (size, shape, margin, texture and surface characteristics). Further identification was carried out by the use of a selective agar medium. Media used for different organisms is given below:

Table I: Media used for different organism

Agar media	Organism
Cystine-Lactose-Electrolyte-Deficient Agar (CLED)	Pseudomonas sp.
Simmons citrate agar with 1% inositol (SCAI)	Klebsiella sp.
Mannitol Salt Agar (MSA)	Staphylococcus sp.
Eosin methylene blue (EMB)	E. coli
MacConkey	Staphylococcus sp. and E. coli
Blood agar	Proteus sp.

For confirmation of E.coli, Klebsiella pneumonia, Staphylococcus aureus and Candida albicans, various biochemical tests were done like hemolytic activity, TSI (triple sugar iron) agar test, sugar fermentation test, indole production test, Methyl Red (MR) and Voges-Proskauer (VP) test.

Ripe fruits of C. sinensis and P. guajava that were free from insect infestation and other kinds of damage were collected from the local market of Lahore. While other plant parts of both plants (leaves, stem bark, roots) were collected from the local gardens of Pattoki. The disc diffusion method was used for carrying out the antimicrobial activity.

RESULTS

A urine sample of both the diabetic and non-diabetic populations was taken. Of these 125 were found diabetic and a further 105 were positive for urinary tract bacteria.

Table II: Distribution of positive urine cultures among samples.

	Number	Positive urine cultures	ASB	UTI
Diabetic	125	75	46	29
Non-diabetic	125	30	12	18
Total number of samples	250	105	58	47

Key: ASB=Asymptomatic Bacteriuria UTI=Urinary Tract Infection

There was a total of 105 samples that were found to have positive urine cultures. Among these positive urine culture samples, 75 were diabetics and 30 were non-diabetics. The bacterial isolate most commonly found among diabetics was E. coli followed by the presence of S. aureus, K. pneumonia, P. aeruginosa, P. vulgaris respectively

Table III: Isolates obtained from Diabetics and Non-Diabetics

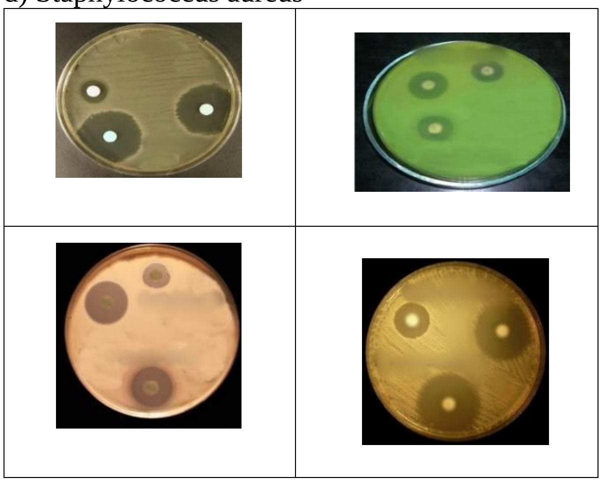
Sr. No	Organism	Diabetics Positive cases	Non-Diabetic Positive Cases
1	Escherichia coli	41	12
2	Staphylococcus aureus	19	7
3	Klebsiella pneumoniae	9	5
4	Pseudomonas aeruginosa	5	6
5	Proteus vulgaris	1	0

MIC of different parts of plant extracts against isolated bacteria from diabetics showed that the highest sensitivity was found against peel, leaves and seed extracts of C. sinensis and P. guajava while the least sensitivity was shown against the stem and root extracts of these plants. The abilities of the extracts were also affected by increasing concentrations; for leaf extracts, increasing concentration (i.e., 2.5mg and 5mg) was directly proportional to the diameter of the zones of inhibition

Table IV: MIC (mm) of different parts of plant extracts against isolated bacteria from diabetics

Plant species/ Solvent	Escherichia coli	Staphylococcus aureus	Proteus vulgaris	Klebsiella pneumoniae	Pseudomonas aeruginosa
Citrus sinensis (Sweet Orange)					
Leaves/Ethanol	15	12	7	11	7
Leaves/P. ether	11	11	5	10	5
Stem/Ethanol	0	0	0	0	0
Stem/ P. ether	0	0	0	0	0
Roots/Ethanol	0	0	0	0	0
Roots/ P. ether	0	0	0	0	0
Peel /Ethanol	20	17	10	13	15
Peel/ P. ether	18	15	10	9	10
Psidium guajava (Guava)					
Leaves/Ethanol	25	29	12	18	15
Leaves/ P. ether	21	26	10	15	13
Stem/Ethanol	7	7	0	10	0
Stem/ P. ether	5	6	0	7	0
Roots/Ethanol	0	12	0	10	0
Roots/ P. ether	0	10	0	9	0
Seed /Ethanol	10	14	7	13	10
Seed / P. ether	9	12	6	10	9

Figure I: Antibacterial sensitivity of P. guajava leaves extract against isolates
a) Escherichia coli
b) Pseudomonas aeruginosa
c) Klebsiella pneumoniae
d) Staphylococcus aureus



DISCUSSION

In this study, out of the 125 diabetic and non-diabetic patients 60% prevalence of bacteriuria was found while in 125 non-diabetics it was 24%. According to one study, the overall incidence of UTI in diabetic participants was significantly higher than in non-diabetic participants. (13.67% vs 6.40%; P=0.004).[21] Another study found that 35/256 (13.67%) diabetic patients had culture-positive UTIs compared to 18/250 (7.2%) non-diabetic patients. Diabetes increased the risk of UTI by two-fold (p = 0.01; odds ratio [OR]: 2.04; confidence interval [CI]: 1.12, 3.71).[22] These findings are consistent with the current study. These increased cases of UTI in diabetics as compared to non-diabetics are because most of the early cases of diabetes and associated UTIs remain undiagnosed. Many studies reported an even higher percentage of isolated *E. Coli* in relation to gender of patients with UTI, 96 (60.0%) were females and 64 (40%) were males.[23] Urinary tract infections are mainly caused by Gram negative bacteria which account for 80–85% and the leading causative organisms are *Escherichia coli* (*E. coli*) (75.5–87% of UTI cases)[23]. Also in this current study, *E. coli* was the most prevalent infection-causing agent *S.aureus* was the second most common culprit of UTI followed by *K. pneumoniae*, *P. aeruginosa* respectively and *P. vulgaris* being the least common microbe isolated. Other than E. *coli* some less prevalent pathogens were found such as Klebsiella oxytoca (2.5%, each) Klebsiella pneumoniae (16.3%), Pseudomonas aeruginosa (5.6%) and Proteus *vulgaris* (1.3%). Similar pathogens observed in various studies. [23] The studies identified *E. coli* (80%) as the most associated uropathogen with UTIs. [24] The pattern of antimicrobial susceptibility of these uropathogens changed with the pathogen and plant part used. E. coli showed similar rates of resistance to roots and stems of guava and sweet orange plant parts. Other uropathogens also showed similar resistance patterns. However, leaf extracts from both plants, as well as guava seed extract and sweet orange peel extract, were found to be the most resistant to these bacterial isolates. Guava leaf ethanolic extracts demonstrated significantly high resistance to all isolates.[6.] Peel of citrus fruits was reported by many researchers for its antimicrobial activities [25] but there are only a few studies that focused on the antimicrobial activity of other parts of citrus such as leaves, stems and a few about roots. The current study used ethanolic extract and petroleum ether extracts from citrus leaves, stems, peel, and roots to study their antimicrobial activity. In the present study, MICs of a few Citrus and guava extracts against all UTI pathogens (S. *aureus*, E. *coli*, Proteus *vulgaris*, Klebsiella *pneumoniae*, Pseudomonas *aeruginosa*) were determined for antimicrobial activities. Among them, the most effective agent was *P. guajava* leaf and the *C. sinensis* peel against all five pathogens and had potent antimicrobial activity. Citrus root and stem extracts showed no antimicrobial activity against the selected bacterial pathogens. The same results were obsereved in a study by Sholeh et al, where roots and stem of Citrus showed no antimicrobial activity against any of the microbe isolated.[9]

In this study P. *guajava* bark and roots extract showed no activity against all five bacterial isolates at any of the concentrations of the extract used, which may either be due to unsuccessful extraction from the bark or that the bacteria are resistant to P. *guajava* bark extract. The difference in activities among the different parts of the same plant is due to variations in the phytochemicals present in different plant parts. Increasing concentrations of the extracts also affect their abilities; as for leaf extracts increasing concentration (i.e, 2.5mg and 5mg) was directly proportional to the diameter of the zones of inhibition.

Overall results of our results shows that guava leaves have maximum potential against bacteria as the results show that all the isolates i.e, E. *coli*, Staph. aureus, Klebsiella pneumoniae, Pseudomoanas aeroginosa and Proteus vulgaris from both diabetic and non-diabetic population showed sensitivity against leaf extracts of Psidium guajava. Furthermore, the extracts in ethanol were found to be more effective rather than ether extracts. There is more need to execute more and more screening of natural products or plant parts so that further phytochemical, pharmacological and in vivo studies may open the possibilities of finding the new clinically effective antibacterial compound against other bacterial-resistant pathogens.

CONCLUSION

From the results, it is concluded that leaves extract of Psidium guajava and Citrus sinensis peel extracts showed effective results against bacterial pathogens and could serve as a good alternate source of antibacterial agents since it is a well-known fact that day-by-day microbes are becoming resistant to common antibiotics. Thus, the present study presents leaves of guava and peel of sweet orange as the drug of choice for the treatment of urinary tract infection (UTI) during diabetes. Further concluding that according to literature guava leaves also have an anti-diabetic effect so it could prove to be the best source of drug used for UTIs in diabetics that would play a dual medicinal role.

Recommendations for Future Research

This research recommends the use of plant parts as antimicrobial agents for UTIs. Diabetics' hygiene should be improved and glycemic control should be maintained as well to reduce UTIs prevalence. Further studies can be conducted for the isolation of microbes in diabetic patients in well-developed areas of Pakistan. Various different plant extract solvents can be used. Other components of Citrus sinensis and Psidium guajava can be used to test the sensitivity against microbes.

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Author's Contribution	
Syeda Aatika Batool	Conceived idea, Study designed, data collection, manuscript
Farheen Ansari	Study design, critically revised all intellectual contents and approved the final version
Sidra Gondal	Study design, data collection, revise and approve the final version
Mahreen Muhmood	Study design, data analysis and interpretation of results, approved the final version
Kainat Asmat	Study design, data collection, revise and approve the final version
All the authors are accountable for validity of data.	

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