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Original article

Effect of traditionally used herb *Pedalium murex* L. and its active compound pedalitin on urease expression – For the management of kidney stone

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ABSTRACT

Pedalium murex L. is a medicinal herb that has been used for the treatment of diseases related to kidney in the traditional system of medicine. The current study aims to study the effect of ethyl acetate extract of *P. murex* (EAEP) and its fractionated compound pedalitin against urease production and *UreC* gene expression in *Proteus mirabilis*. The selected reference strain *Proteus mirabilis* (MTCC 425) and the isolates culture of *Proteus mirabilis* were subjected to study the antibacterial efficacy of *P. murex*. Expression analysis of *P. mirabilis* urease gene was successfully done by QPCR. The ethyl acetate extract effectively inhibit the reference *Proteus mirabilis* and bacterial isolates of *Proteus mirabilis in the clinical samples studied*. EAEP has showed more potent activity (56.7%) against urease enzyme and pedalitin also exhibited potent activity (30.1%). Using qPCR, the expression of *UreC* gene of *P. mirabilis* was controlled by EAEP and also its bioactive compound pedalitin. The present study clearly demonstrated the potency of *P. murex* in controlling the growth of pathogenic *P. mirabilis* and to control the expression of urease enzyme production as well as to restrict the urease gene expression in *P. mirabilis*.

1. Introduction

Urinary tract infection (UTI) is a severe health disease with high complication because it is related to antibiotic resistance and threatening to health throughout lifetime (Thulasi and Amsaveni, 2012). According to the World Health Organization (WHO), urinary diseases cause death of almost 85,000 people in the world per year. Among different types of kidney stones, struvite stone is the second major type of stone and is referred as an infection stone which is composed of Magnesium Hydrogen Phosphate Tetrahydrate - [$MgHPO_4$- 3(H_2O)] and Ammonium Magnesium Phosphate Hexahydrate (AMPH) - [(NH_4) $MgPO_4$- 6(H_2O)]. Struvite stone may raised by increasing super-saturation of different elements present in the urine that would cause the stone formation (Worcester and Coe, 2010). The stone formation occurs mainly based on mineral accumulation followed by nucleation, growth of crystal, crystal aggregation as well as crystal retention (Kaleeswaran et al., 2019). Some uropathogens such as *Proteus mirabilis, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Mycoplasma* species are also involved. If it is untreated, it can damage the kidneys and can become end-stage renal disease (ERD). Current management of kidney stone, creates side effects or recurrence of stone because of the high risk factors; we look back for lesser or without side effects treatment with a herbal medicinal plant.

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Indian system of medicine recommends medicinal plants as alternative medicine for the treatment of kidney stone and also several diseases. Based on traditional healers, the plant *Pedalium murex* L. was used for the dissolution and prevention of kidney stone formation. Further, it is used for the treating ailments like incontinence of urine, gonorrhoea, promote lochial discharge, antibilious agent, dysuria and control white discharge. Moreover, the whole plant parts could be used for the treatment of urinary problem, diuretic, male fertility disorder and leucorrhoea. Likewise, fruit and dried fruit were used to recover the diseases such as diabetes, demulcent, gonorrhoea, aphrodisiac, antispasmodic property and incontinence of urine, strangury and urinary calculi. Some diseases like ulcer, dysuria, splenic enlargement and diarrhoea, gonorrhoeal rheumatism, aphrodisiac and demulcent were treated by using leaves (Al-Dhabi et al., 2015; Barathikannan et al., 2016; Al-Dhabi and Arasu, 2016) The seed of this plant was used as a treatment of leucorrhoea, urinary tract disorder, joint pain, lumbago, bladder troubles and gonorrhoea (Cuong et al., 2017; Elango et al., 2017, 2016a, 2016b). By using, stem part of *P. murex* used for the treatment of spermatorrhoea, dysuria, ardour urinae and gonorrhoea (Imran et al., 2015; Glorybai et al., 2015; Fowsiya et al., 2016; Haritha et al., 2016). It has been also used for the veterinary disease treatment. Each plant parts were used as medicine for the curable of various diseases (Table 1). Thus, the plant has lot of active constituents but none of the work has been implemented for treating the struvite stone (see Table 2).

Instead of using allopathic medicine, researchers isolate the active components from medicinal plants for disease treatment (Rahmanet al., 2011; Islam et al., 2015; Helan et al., 2016; Ilavenil et al., 2017; Park et al., 2016a, 2016b). In our research, based on the evidence of previous literature, the extracted bioactive compound could be selected by the colour and its melting point, which we have selected the active compound, it may be as pedalitin (Park et al., 2017) Further, the characterization of bioactive compound is still in laboratory testing for the confirmation as pedalitin. With the assumption and identification of the compound pedalitin, further were used for the inhibitory activity against virulence factor urease and its gene of expression especially *UreC* gene in *P. mirabilis* by biochemical method, gene expression and molecular docking studies.

Table 1
Pedalium murex L.: Plant description and its medicinal value.

S. No	Description	Prediction of plant	
1.	**Scientific Name**	*Pedalium murex* L.	
2.	**Family**	Pedaliaceae	
3.	**Vernacular name**	Yaanainerinji	
4.	**Parts used**	Medicinal uses	References
	Whole plant	Urinary problem, urinary calculi, urinary troubles, dieurtic, male fertility disorder, leucorrhoea,	Imran et al., 2015
	Fruits	Diabetes, demulcent, antispasmodic and aphrodisiac, Gonorrhoea	Imran et al., 2015
	Dried fruits	Incontinence of urine, urinary calculi, Strangury	Imran et al., 2015
	Leaves	Ulcers, dysuria, Bone fracture, diarrhea, splenic enlargement, diabetes, Gonorrhoeal rheumatism, Aphrodisiac, Demulcent	Imran et al., 2015
	Root	anti-bilious, calm body heat, virility, Pousthik	Imran et al., 2015
	Seed	Leucorrhoea, urinary tract disorders, diuretic property, joint pain & lumbago, bladder troubles and gonorrhea	Imran et al., 2015
	Stem	Spermatorrhoea, Dysuria, Ardorurinae, Gonorrhoea	Imran et al., 2015

Among several microbes, *Proteus mirabilis* is an extremely pathogenic bacteria and it is the main reason for most complicated UTI such as the development of staghorn stone in kidney and blockage of urinary tract (Al-Duliami et al., 2011; Surendra et al., 2016a, 2016b, 2016c). It forms infection in the upper urinary tract, sequentially it can causes diseases like urolithiasis, cystitis and acute pyelonephritis and occasionally found in wound infections, bacteremia, septicemia, neonates or infants meningitis and rheumatoid arthritis (Hasan and Al-Azawi, 2011).

Proteus mirabilis has several virulence factors like adhesions, hemolysin, urease, lipopolysaccharide endotoxins, swarming motility and proteases (Armbruster and Mobley, 2012; Gurusamy et al., 2019; Rajkumari et al., 2019). Among these virulence factor, Urease is the main factor and it is the main reason for the development of urinary stone by the pathogeneticity of *P. mirabilis*. This enzyme highly mediates the formation of ammonia and carbondioxide from urea which in turn, increases the urine pH to deposit the crystalline minerals in the urinary tract that develops into kidney stone (Fig. 1). It is a multimeric nickel-metalloenzyme, which is programmed by urease gene cluster (*UreDABCEFG*) called as urea-inducible genes. Among this gene cluster, *UreC* is the main reason for the high pathogenicity of *P. mirabilis*, because it yields high amount of urease. Our aim is to suppress the *UreC* gene expression using *P. murex* plant extract and its bioactive compound pedalitin.

2. Materials and methods

2.1. Plant specimen

Whole plants of *Pedalium murex* (L.) was collected from Thanjavur, Tamil Nadu, India and was authenticated by the Director of the Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph College, Tiruchirappalli. The plant was assigned a voucher number RHPM SR 001.

2.2. Solvent extraction of plant

The plant sample was completely dried under shade, ground to fine powder by an electrical mixer and sieved through a 20 μ mesh sieve. The powdered sample was extracted using soxhlet apparatus with ethyl acetate, filtered through and concentrated at 45 °C using rotary vacuum evaporator under reduced pressure until it becomes a thick paste. Finally, it was yielded 15.1% w/w in terms of dried material and it was kept at 4 °C in a refrigerator an air tight glass bottle for this study (Chopra et al., 1992).

2.3. Antibacterial assay

2.3.1. Isolation, identification and antibiotic sensitivity test

Most common UTI pathogen *Proteus mirabilis* was isolated from clinical samples and used for this study. The UTI patients urine samples were collected from National pharma hospital, Thanjavur and reference strain *Proteus mirabilis* from MTCC 425. Identification of pathogenic bacterial strains was done by colony morphology and it was compared with reference strain. Antibiotic sensitivity test for the bacterial strain was done by the Kirby-Bauer's disc diffusion method. Antibiotic susceptibility test was performed following Clinical Laboratory Standard Institute guidelines (CLSI, 2011).

2.3.2. Antimicrobial activity of EAEP against P. mirabilis

The antibacterial efficacy of EAEP against the clinical samples were analysed by disc diffusion method (Bauer et al., 1966; Baba and Malik, 2015).

Table 2
Urease enzyme assay using Weatherburn method.

S. No	Inhibitory agents	Concentration ml	Extract ml	Urease enzyme from *P. mirabilis* ml	Phosphate buffer ml	Urea ml	Phenol reagent ml	Alkali reagent ml
1.	EAEP	1%	0.1	0.2	1.2	0.5	1	1
2.	Pedalitin	1%	0.1	0.2	1.2	0.5	1	1
3.	Thiourea	1%	0.1	–	1.2	0.5	1	1

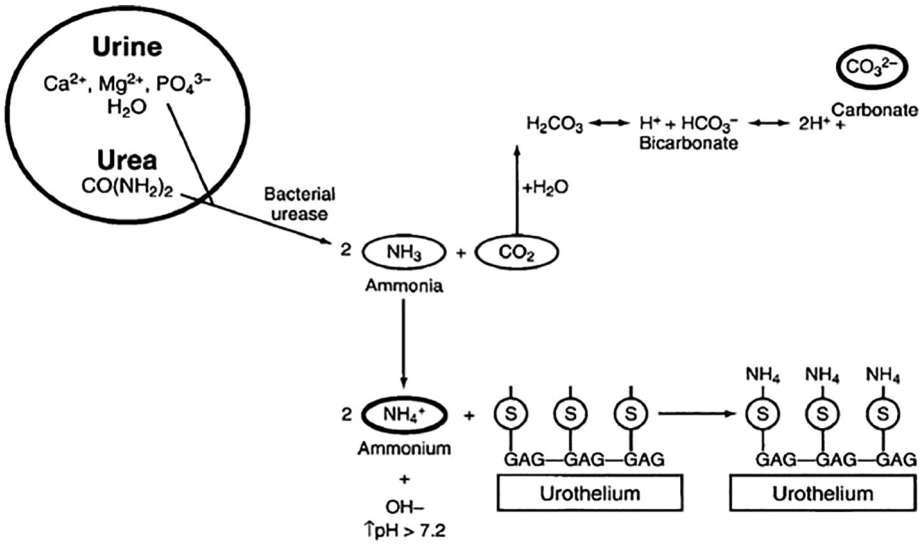

Fig. 1. Chemical process of urease producing bacteria; S = negatively charged sulphate group, GAG = glycoaminoglycan layer, = components of urine.

2.4. Urease inhibitory activity

2.4.1. Extraction of urease enzyme

For the production of urease, the overnight culture of *P. mirabilis* MTCC 425 of 50 μl cultivated in Mueller Hinton Broth (MHB). Then they were transferred into sterile MHB of 10 ml and then it was incubated for 18 hrs at 37 °C with even shaking. After that, the cells were deposited as pellet by centrifugation at 1258 rpm for 15 min (4 °C). With the solution of 10 mM K_2HPO_4 the pellet was washed by three times and again immersed in 2 ml of the same solution. Then, it was sonicated for 90 sec with 0.5 cycles using an ultra sonicator at 100% amplitude with an ice container for releasing urease from bacteria. Finally, the bacterial lysate was collected and used for urease activity assay (Ranjbar-Omidet al., 2015).

2.4.2. Inhibitory effect of urease enzyme – Weatherburn method

For the inhibition activity of urease enzyme, EAEP and pedalitin compound were subjected in indophenol method (Weatherburn, 1967). The assay solution was contained with a bacterial urease solution (0.2 ml) mixed with 0.1 ml of extract along with 1.2 ml of phosphate buffer (pH 8.2) and it was incubated at 30 °C for 5 min. After incubation, aliquots were taken and added to 0.5 ml (66 mM) of urea and the whole sample was incubated for 20 min. Thiourea was used as the standard inhibitor for urease activity.

2.5. qPCR – gene expression analysis

The *Proteus mirabilis* strain (MTCC 425) was grown in LB broth to delay logarithmic phase and then the cells were collected and kept at 20 °C. The total RNA was extracted from the bacteria using RNeasey protects bacteria mini kit (Qiagen USA). Then, DNA in the sample was digested by RQ Dnase. The total RNA was quantified using spectramax i3 with spectral Drop Micro- Volume Microplate (Molecular devices, USA). The RNA was reverse transcribed with cDNA synthesis kit (iScriptcDNA synthesis kit, Biorad).

Quantitative PCR was performed with CFX 96 PCR model (Applied Biosystems, USA). Target gene expression level was quantified using SYBR green based qPCR in 10 μl reactions containing 5 μl Power SYBR Green Master Mix (Biorad, USA), 1 μl cDNA, 1 μl 10 p mole forward (FP: CCG GAA CAG AAG TTG TCG CTG GA) and reverse primers (RP: GGG CTC TCC TAC CGA CTT GAT C) and 3 μl DEPC water. The target gene expression was calculated by the $2^{-\triangle\triangle CT}$ (Livak method). The gene expression was normalised with housekeeping gene*rpoA*(RNA polymerase A).

3. Results

3.1. Antibacterial activity

3.1.1. Antibiotic sensitivity test of P. mirabilis

The antibiotic profiles of pathogenic bacteria were determined using specified antibiotic discs Hexa UTI 5 containing different antibiotics. Isolated gram negative bacteria, *P. mirabilis* were more resistant to Amoxyclav, Ampicillin, Ciprofloxacin, Co-Trimoxazole, Nitrofurantoin, Norfloxacin in sample 5 and very sensitive in sample 1 against these antibiotics in the disc. The details of individual antibiotics resistant profiles of individual bacteria are represented (Table 3).

3.1.2. Antibacterial test of plant extracts

From the results, it was proved that the tested extract has significant antibacterial potency against *P. mirabilis* strains from clinical isolates and its reference strain from MTCC 425 (Table 4). It was observed that EAEP showed highest antibacterial activity against UTI bacteria which causes severe infection in patients.

Table 3
Antibacterial activity of standard antibiotics against *Proteus mirabilis* (Himedia - Hexa UTI 5) (Zone of inhibition in mm (Mean ± S.D)*).

S. No	*Proteus mirabilis*						
	Samples	Antibiotics in disc (Zone of inhibition in mm (Mean ± S.D)*)					
		Norfloxacin (NX-10 μg)	Ciprofloxacin (CIP −5 μg)	Ampicillin (AMP − 10 μg)	Co-Trimoxazole (COT − 25 μg)	Nitrofurantoin (NIT − 300 μg)	Amoxyclav (AMC − 30 μg)
1.	**I**	33.3 ± 6.02	38.3 ± 2.88	16.0 ± 6.0	12.6 ± 12.0	00	00
2.	**II**	25.0 ± 2.64	26.0 ± 5.50	00	00	00	00
3.	**III**	22.6 ± 9.29	33.6 ± 4.16	00	00	00	00
4.	**IV**	26.6 ± 1.52	34.6 ± 1.52	00	11.6 ± 7.0	00	00
5.	**V**	00	00	00	00	00	00
6.	**VI**	17.3 ± 11.0	18.3 ± 14.4	00	00	00	00
7.	**VII**	25.3 ± 4.04	29.6 ± 0.57	12.3 ± 2.51	00	00	10.0 ± 1.0
8.	**VIII**	31.0 ± 1.73	32.3 ± 2.51	6.66 ± 5.7	00	00	00
9.	**IX**	16.3 ± 10.1	18.6 ± 10.9	0	00	00	00
10.	**X**	22.0 ± 7.54	21.6 ± 10.0	0	00	7.66 ± 2.08	00

3.2. Inhibitory effects of urease using EAEP and bioactive fractioned compound (Pedalitin)

Urease inhibitory activity of EAEP (0.1 ml) and pedalitin (0.1 ml) was depicted in Table 5. The activity was compared with Thiourea (80 mg/ml) which was used as standard. The urease inhibition of standard Thiourea was 96.3%. EAEP extract was showed the maximum urease activity (56.7%) with considerable value of inhibition and the pedalitin compound showed 30.1% of inhibitory activity.

3.3. qPCR – Gene expression analysis

Expression of urease gene (*UreC*) in *P. mirabilis* was studied and shown in the Tables 6–8. In this analysis, mRNA was extracted from *P. mirabilis* and reverse transcribed. Furthermore, the target gene expression was quantified by qPCR. From the evidence of linear regression correlation, the expression of *UreC* was significantly responded by the treatment of EAEP and Pedalitin. The regression calculations of qPCR was showed significant activity in EAEP treated group (r^2 = 0.62 & P = 0.04) than the pedalitin treated group (r^2 = 0.46 & P = 0.03). Moreover, the results were showed that the treatment with EAEP and pedalitin significantly down regulated the expression of *Ure*CmRNAas compared to control group. Overall results of this study, the EAEP was showed more effect on the urease gene expression and moderate effect was noted inpedalitintreatment.

Table 5
Inhibitory activity of *Pedalium murex* L. against urease enzyme.

Test extracts	Urease inhibitory activity (in percentage)
Ethyl acetate (5 μl)	56.7%
Pedalitin compound (5 μl)	30.1%
Thiourea (Standard) (80 mg/ml)	96.3%

4. Discussion

Universally, the bacterial infections are an important cause of morbidity and mortality. The exploration for antimicrobials from plant source has expected much attention and efforts to discover the compounds that can perform as right antimicrobial agent to change the synthetic ones (Sen and Batra, 2012). In most of the common cases, the microbes do not involve directly to cause disease. It allows the bacteria to overcome the natural protective mechanisms of the body through the damage of the skin, leading to infections (Ejaz et al., 2014). In the present situation, the use of medicinal herbs and conventional traditional drugs is quiet economical when compared to modern medicine. It is mandatory to inspect and authenticate various indigenous drugs along with better understanding of their biological and pharmacological properties (Kelmansonet al., 2000; Roopan et al., 2019; Valsalam et al., 2019a, 2019b).

Phytocompounds can serve as a prototype to develop less lethal and efficient drug for controlling the development of microbes (Sen and Batra, 2012). Principally, it involved in the separation and discovery of the secondary metabolites extracted from the plants and used as the active ingredients in medical preparations (Aiyegoro and Okoh, 2009). Nowadays, most of the people interested to consume drugs with safe, effective from natural products as extracts or plant oils that is alternative to the commercial synthetic medicine. These antimicrobial drugs have enormous potentially effective therapeutic value which involve in the treatment of infectious disease caused by microbes (Zablotowicz et al., 1996).

Among the various diseases affecting human population, kidney stone stands third. It may be infectious or non-infectious, mainly originating from metabolic disorders or through some unknown changes and also with urinary tract infections (Kunin, 1997). Bacterial infection can disturb any part of urinary tract (Stamm and Norrby, 2001) and if untreated they make much damage. It may cause serious medical consequences such as extreme obstruction, hydronephrosis, infection and haemorrhage in the urinary tract system. It is reported that these infections are more common in women than men (Al-Jiffriet al., 2011). The main causative agents for the stone formation was screened by the presence of different elements by means of increasing super-saturation of urine (Worcester and Coe, 2010; Ghelaniet al., 2016).

Table 4
Assessment of Antibacterial activity, MBC and MIC of EAEP against clinical isolate *P. mirabilis* and its reference strains (Zone of inhibition in mm) (Values in μg/ml).

S. No	*P. murex*EXTRACT	Organisms								
		Clinical samples (Zone of inhibition in mm (Mean ± S.D)*)						Standard bacteria (Zone of inhibition in mm (Mean ± S.D)*)		
		PM5	MBC	MIC	PM1	MBC	MIC	*P. mirabilis* (MTCC 425)	MBC	MIC
1.	**EAEP**	14.3 ± 1.00	125	125	18.6 ± 1.52	62.5	125	20.6 ± 1.15	125	62
2.	**DMSO**	0	0	0	0	0	0	0	0	0

Table 6
Effect of normal treatment on gene *UreC* of *Proteus mirabilis* using qPCR.

NO	Treatment group	Target urease			House Keeping	References				Mean	SEM
1.	Control	16.76	E11	SYBR	16srRNA	14.75	2.01	0	1.00	0.99 ± 0.12	0.0446014
2.	Control	16.87	D11	SYBR	16srRNA	14.86	2.02	0.01	1.0		
3.	Control	16.52	D12	SYBR	16srRNA	14.79	1.73	−0.29	1.22		
4.	Control	16.57	F11	SYBR	16srRNA	14.83	1.75	0.02	0.99		
5.	Control	16.75	C11	SYBR	16srRNA	14.81	1.94	0.19	0.87		
6.	Control	16.87	A11	SYBR	16srRNA	14.91	1.96	0.02	0.98		
7.	Control	16.73	B11	SYBR	16srRNA	14.87	1.86	−0.11	1.08		
8.	Control	16.71	H11	SYBR	16srRNA	14.53	2.18	0.33	0.80		

Table 7
Effect of EAEP treatment on gene *UreC* of *Proteus mirabilis* using qPCR.

S. No	Treatment Group	Target urease			House Keeping	References				Mean	SEM
1.	EAEP	18.19	G10	SYBR	16srRNA	15.15	3.04	1.04	0.49	0.33 ± 0.09	0.0341616
2.	EAEP	18.97	F10	SYBR	16srRNA	15.08	3.89	1.88	0.27		
3.	EAEP	18.44	*H*10	SYBR	16srRNA	14.30	4.14	2.41	0.19		
4.	EAEP	18.30	B10	SYBR	16srRNA	14.73	3.56	1.82	0.28		
5.	EAEP	18.44	A10	SYBR	16srRNA	14.84	3.60	1.66	0.32		
6.	EAEP	18.72	C10	SYBR	16srRNA	15.03	3.69	1.73	0.30		
7.	EAEP	18.25	E10	SYBR	16srRNA	15.12	3.12	1.27	0.42		
8.	EAEP	18.02	D10	SYBR	16srRNA	14.56	3.46	1.28	0.41		

Table 8
Effect of Pedalitin treatment on gene *UreC* of *Proteus mirabilis* using qPCR.

S. NO	Treatment group	Target urease			House Keeping	References				Mean	SEM
1.	Pedalitin	17.69	D12	SYBR	16srRNA	14.78	2.91	0.90	0.53	0.62 ± 0.11	0.0413272
2.	Pedalitin	17.17	E12	SYBR	16srRNA	14.81	2.36	0.34	0.79		
3.	Pedalitin	17.47	F12	SYBR	16srRNA	14.91	2.55	0.83	0.56		
4.	Pedalitin	17.66	G12	SYBR	16srRNA	14.98	2.69	0.94	0.52		
5.	Pedalitin	17.65	C12	SYBR	16srRNA	14.77	2.89	0.95	0.52		
6.	Pedalitin	17.87	H12	SYBR	16srRNA	15.06	2.80	0.84	0.56		
7.	Pedalitin	17.01	A12	SYBR	16srRNA	14.81	2.20	0.34	0.79		
8.	Pedalitin	17.77	B12	SYBR	16srRNA	15.04	2.73	0.54	0.69		

Presence of broad multiplicity of natural products in the world has been used as a drug against various diseases. Further, it has been examined by the researcher for the preventive and management of diseases, especially for the antibiotic resistance pathogens. Based on the traditional knowledge, the plant *P. murex* was used for the treatment of UTI, particularly caused by *P. mirabilis*, which was the main causative factor for the urinary tract infection, in turn it develops the struvite stone or infection stone. In the past work, the *P. murex* was showed highest inhibitory activity against various UTI bacteria such as *E. coli*, *P. mirabilis*, *B. cereus*, *S. aureus*, *B. licheniformis* and *S. typhi* (Kaleeswaran and Ramadevi, 2016). The reason behind this inhibitory activity is the presence of some active compounds which may be responsible for providing resistance against the development of infection caused by the microbes.

P. mirabilis is recognized by its capability to colonize the virulence factors. The virulence factor, urease enzyme mediate the conversion of urea into ammonia and CO_2 which alter the pH that deposit the polyvalent ions in the urine termed as struvite stone. It increases the colonization in catheter, bacterial observance, development of biofilm incrustation and also increase the swarmer cells, in turn, it facilitates into the formation of bacterial infection (Al-Mayahi, 2017). It may cause serious medical consequences such as extreme obstruction, hydronephrosis, infection and haemorrhage in the urinary tract system. The immune system in human cannot eliminate because of the bacterial capacity of immune evasion. Thus, suppressing the expression of virulence factors to facilitate the pathogenicity of the bacterium and ease the elimination for the host immune system to overcome infection (Fernebro, 2011). For this purpose, urease inhibitors had been extracted from some plants such as *Allium ursinum, Hyssopus officinalis, Potentilla argentea, Salvia sclarea, Yucca filamentosa* and *Fagonia arabica* (Amin et al., 2013; Modolo et al., 2015). We studied *P. murex* plant that has not been analyzed yet for its inhibitory activities of urease in *P. mirabilis* in order to find out its effect on the factor responsible for colonization and virulence capacity of the bacterium.

The flavonoid compound Pedalitin present in *P. murex* may be responsible for the urease inhibitory activity of *P. mirabilis*. The result showed that the EAEP and Pedalitin have more potent activity against urease. It was also proved from previous work in different plant with various organisms (Khan et al., 2014; Ali et al., 2015). The plant *Hibiscus schizopetalus* showed urease inhibitory activity as 55.5% (Zahid et al., 2014). A stronger urease activity was also reported in *Sambucus ebulus* and *Rheum ribes* extracts using *in-vitro* method (Nabati et al., 2012). Five different plants such as *Matricaria disciforme*, *Nasturtium officinale*, *Punica granatum*, *Camelia sinensis and Citrus aurantifolia* also showed potent inhibitory activity against urease enzyme of Horse gram (Biglar et al., 2012).

We analysed the urease producing gene *UreC* expression in *P. mirabilis* using specific primer sequences which is responsible for the expression of gene. Mobley and Chippendale (1990) reported that the provisory of this *P. mirabilis* highly produced the urease enzyme and it has to detect the gene by molecular identification. Among this gene clusters, a broad distribution of virulence factors *UreR* and *UreC* with *P. mirabilis* was identified by the researchers Mobley and Chippendale (1990) and MacFaddin (2000). Further, in our research, we were mainly focused on the mRNA expression of *UreC* in control and treated bacteria because it is very important gene present in the *P. mirabilis* that produces urease abundantly.

Urease is an apoenzyme which is composed as a trimeric complex with trimer *URE-ABC*. For the activation of this apoenzyme, it requires a nickel ion which is located in *UreC* in the metallocenter region (Sambrook and Rusell, 2001; Alatrash and Al-yasseen, 2017) and it is larger subunits present in *P. mirabilis*. Therefore, we quantified expression level of urease producing gene in *P. mirabilis*. Our result suggested that EAEP and pedalitin might inhibited the expression of virulence factor especially *UreC* in *P. mirabilis*. This is the first information that describes the inhibitory effect on *UreC* by the treatment of *P. murex* extract and also by the lead compound pedalitin. It may be very useful to pharmaceutical industries for developing a new strategy to control or prevent the kidney stone formation by *P. mirabilis* and other bacteria, particularly against struvite stone.

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