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In vitro antibacterial activity and the minimum inhibitory concentration of aqueous seeds extract of *Cucumis melo* L. grown in Sri Lanka on common urinary tract infective bacteria

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Abstract
In this study, *in vitro* antibacterial activity of aqueous seeds extract of *Cucumis melo* L. grown in Sri Lanka was evaluated on common urinary tract infective bacteria (*E. coli, S. aureus, P. aeruginosa, P. mirabilis and E. faecalis*). Disc diffusion assay and the macro broth dilution method were used to determine the antibacterial activity and the minimum inhibitory concentration (MIC) respectively. Gentamicin and ampicillin were used as the positive controls. The results showed that seeds extract exhibited concentration dependent t marked antibacterial activity against uropathogens at all the concentrations (500 µg/ mL, 1000 µg/ mL and 2000 µg/ mL). Statistical analysis revealed that the results were equipotent to gentamicin and ampicillin which is a novel finding. The highest antibacterial activity was shown against *E. coli* at 2000 µg/ mL (22.0±2.64 mm). Least inhibition zones were showed by *E. faecalis*. The highest MIC was shown by *E. faecalis* (1000 µg/ mL) and the least by *P. aeruginosa* (125 µg/ mL). The results of qualitative phytochemical analysis revealed the presence of alkaloids, phenols, saponins, flavonoids and tannins. Therefore, the extract is likely to mediate its antibacterial activity by synergistic mechanisms. In addition, the results scientifically justified the claim made by Sri Lankan traditional and folk medicine. Findings also indicated the potential of developing a drug for urinary tract infections based on the seeds of *Cucumis melo* L.

Keywords: Urinary tract infections, *Cucumis melo* L., antibacterial, uropathogens, minimum inhibitory concentration, phytochemical analysis

1. Introduction
Urinary tract infections (UTIs) are the most frequently encountered bacterial infections which are responsible for higher rate of morbidity and mortality throughout the world [1]. They are classified according to the site of infection and can be either asymptomatic or symptomatic [2]. The ubiquity of UTI increases with age in both genders with a 50:1 female to male ratio [3]. The economic impacts of UTI are high due to medical costs and nonmedical costs associated with transportation, sick days, and morbidity [2].
Urinary tract infections are often treated with antibiotics that have been used for the last seven decades to treat infections, where they either kill or cease the growth of microbes [4]. However, due to indiscriminate and overuse, pathogenic organisms have developed antibiotic resistance. Though UTIs often self-recover and can be treated with antibiotics, they repeatedly reoccur due to their ability to breach, reproduce and remain within the host epithelial cells. This complexity and rise in antibiotic resistance in uropathogens emphasize the need of alternative remedies [5]. Screening of medicinal plants to develop new drugs with improved safety and efficacy has a long history of use throughout the world [6]. Medicinal plants consist of active constituents and secondary metabolites which make them too complex for bacterial resistance to occur [7]. One such medicinal plant is *Cucumis melo* L. which belongs to the family Cucurbitaceae. It is an annual tendril climber grows in tropical and sub-tropical areas of the world [8]. *C. melo* is distributed in Sri Lanka, India and Malaysia. It is known as "Kekiri" in Sinhala [9] Vellarikkai in Tamil, Chinese white cucumber and Oriental pickling melon in English [10]. *C. melo* has rough, long and angular stems with hairy ridges. Flowers are yellow in color and are monoecious. Leaves are wide and 7.5-11.2 cm long with 3 or 5 acute lobes. Petioles are bulky, twisted and rough with bristly hairs. Fruit is oval or round in shape, slightly trigonal in section, thickly striped with dark and light green, solid and fleshy. Seeds are numerous in number, smooth, horizontal and almost oval in shape [9]. *C. melo* is known to possess many medicinal properties such as analgesic, anti-oxidant, anti-inflammatory,

anti-platelet, free radical scavenging, anti-cancer, anti-ulcer, diuretic, hepato-protective, anti-fertility, anti-microbial, anti-helminthic and anti-diabetic [8]. Phytoconstitiuents of *C. melo* responsible for their particular medicinal properties include carbohydrates, amino acids, fatty acids, glycolipids, phospholipids, β-carotenes, flavonoids, terpenoids, chromone derivatives, ascorbic acid, volatile components and various minerals [8]. In general, environmental factors such as temperature, humidity, pH of the soil, soil fertility, irrigation and rainfall affect the growth of a plant [11]. Due to these variations, the quality and the quantity of phytoconstituents of a particular plant grown in different countries may have different properties. Therefore, Sri Lankan variety *C. melo* may also have variations in phytoconstituents which might leads to different therapeutic properties. In Sri Lanka, seeds of *C. melo* is used in traditional and folk medicine for the treatments of urinary calculi and other urinary complaints [9]. However, as yet, validity of this claim is not scientifically validated or refuted. Accordingly, this current study was aimed at investigating *in vitro* antibacterial activity and the minimum inhibitory concentration of aqueous seeds extract of *C. melo* grown in Sri Lanka on common urinary tract infective bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Enterococcus faecalis*). In addition qualitative phytochemical profile of *C. melo* aqueous extract was analyzed to determine its phytoconstituents.

2. Materials and Methods

2.1. Collection and authentication

Whole matured plants with flowers and fruits were collected from a vegetable farm at Rajanganaya in North Central Province, Sri Lanka (GSI 8º 3’ 43.2” or 8.062°North and 80º 15’ 3.6” or 80.251°East), in July 2017. The plant materials had been identified and authenticated by a botanist at the National Herbarium, Peradeniya, Sri Lanka.

2.2. Preparation of aqueous seeds extract of *Cucumis melo* L.

Fruits with mean weight of 550 g were used for the seed collection. Fruits were washed using tap water and were cut to obtain undamaged seeds. Seeds collected were washed with tap water followed by distilled water. Washed seeds were air dried in shade for 2 days and were ground to a fine powder using a mechanical blender. Powder obtained was stored at room temperature (25 ºC) in an air tight, sterile container protected from sunlight. *C. melo* aqueous seeds extract was obtained by traditional boiling method. Seeds powder of 60 g was weighed and added to 1920 mL of distilled water. Solution was boiled slowly for 3.5 hours until the final volume reached 240 mL. The prepared aqueous extract was left for cooling and then was added to sterile centrifuge tubes. Dry mass of the prepared aqueous extract was obtained by freeze dry method [12] and was stored at 4 ºC in the freezer compartment of the refrigerator until use for the experiment.

2.3. *In vitro* antibacterial assay

Antibacterial activity of freeze dried *C. melo* seeds extract was evaluated using Kirby-Bauer disc diffusion method against *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853), *P. mirabilis* (ATCC 12453) and *E. faecalis* (ATCC 29212) [15]. Concentrations of 500 µg/ mL, 1000 µg/ mL and 2000 µg/ mL of the freeze dried sample were used to determine the antibacterial activity (n=3). Distilled water was used as the negative control while ampicillin (10 µg/ mL) was used as the positive control for *E. faecalis* and gentamicin (10 µg/ mL) was used as the positive control for *E. coli*, *S. aureus*, *P. aeruginosa* and *P. mirabilis.* Sterilized filter paper discs (Whatman 4) of diameter 6 mm were impregnated with 20 µl of prepared concentrations and controls. Discs were incubated at 35 °C for 18-24hours and were immediately used for antibiotic sensitivity test [13]. Suspensions of each test organisms were adjusted to 0.5 McFarland turbidity standard. The surface of the Muller Hinton Agar plate was streaked in three directions rotating at 60°. Impregnated discs were placed aseptically at appropriate places on the inoculated plates with specific bacteria. Plates were kept 15minutes at room temperature (25 ºC) for pre diffusion and then were incubated at 35 °C for 24hours. The diameter of inhibition zones were measured using a ruler. The experiment was carried out in triplicates and the diameter of inhibition zones for the extract against each test organism were measured and recorded in mm [14].

2.4. Evaluation of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the aqueous seeds extract of *C. melo* was determined by macro broth dilution method using Mueller–Hinton broth as the medium [15]. Stock concentration of 4000 µg/ mL was prepared using freeze dried sample and was subjected to two-fold dilution preparing a range of 4000 µg/ mL to 7.81 µg/ mL. Bacterial suspension adjusted to 0.5 McFarland standard was inoculated. MHB with only bacterial suspension was used as the positive control, while MHB alone was used as the negative control. Entire procedure was carried out for all the test organisms. Tubes were incubated at 35 °C for 24 hours and were interpreted to determine MIC. MIC was interpreted as the lowest concentration of the extract that did not show any visible growth of bacteria when compared to control tubes [15].

2.5. Statistical analysis of results

The results are given as mean ± S.D. SPSS version 23 software was used in interpreting the statistical data. Statistical comparisons were made using Mann-Whitney U Test considering $p<0.05$ as the level of significance. Dose dependencies were evaluated using linear regression analysis.

3. Results

In vitro antibacterial activity of aqueous seeds extract of *C. melo* and standard antibiotics against test organisms are shown in Table 1. and Table 2. As shown, the highest susceptibility was shown against *E. coli* with maximum inhibition zone of 22.0±2.64 mm at 2000 µg/ mL. The least susceptibility was shown against *E. faecalis* with zones of 9.3±0.57 mm, 11.3±1.15 mm and 14.6±0.57 mm at 500 µg/ mL, 1000 µg/ mL and 2000 µg/ mL respectively. The results were compared with the standard antibiotics gentamicin (10 µg/ mL) and ampicillin (10 µg/ mL). Statistical analysis using Mann-Whitney U Test revealed that the test values had no significant difference as p values were greater than 0.05 which indicated that the results were equipotent to standard antibiotics used. The results of linear regression evaluated that the antibacterial activities were dose dependent. Minimum inhibitory concentrations of *C. melo* aqueous seeds extract are shown in Table 3. MIC of *C. melo* was found to be in the range of 125 µg/ mL to 1000 µg/ mL. *E. faecalis* showed a maximum MIC value of 1000 µg/ mL and *P. aeruginosa* showed a least MIC value of 125 µg/ mL. *E. coli* and *P.*

mirabilis showed a similar MIC value of 500µg/ mL, while *S. auerus* showed a MIC of 250 µg/ mL. The results of the qualitative phytochemical analysis of Sri Lankan variety *C. melo* aqueous seeds extract showed the presence of alkaloids, flavonoids, phenols, saponins, tannins and the absence of diterpenes and glycosides (Table 4.).

Table 1: *In vitro* antibacterial screening results of *Cucumis melo* L. aqueous seeds extract (Mean ± S.D.)

Test organism	Zones of inhibition (mm)		
	500 µg/mL	1000 µg/mL	2000µg/mL
E. coli	13.30±1.52	19.00±3.60	22.00±2.64
S. aureus	14.00±2.64	17.60±2.08	19.60±2.08
P. aeruginosa	12.00±1.00	14.00±1.73	16.30±0.57
P. mirabilis	14.30±1.52	16.00±0.00	18.60±1.15
E. faecalis	9.30±0.57	11.30±1.15	14.60±0.57

Table 2*: In vitro* antibacterial screening results of standard antibiotics (Mean ± S.D.)

Test organism	Gentamicin (10µg/ mL)	Ampicillin (10µg/ mL)
E. coli	23.30±2.88	21.60±2.88
S. aureus	24.00±1.73	
P. aeruginosa	19.30±0.57	
P. mirabilis	22.30±2.51	
E. faecalis		

Table 3: Minimum inhibitory concentration (MIC) values of *Cucumis melo* L. aqueous seeds extract

Test organism	MIC value (µg/ mL)
E. coli	500
S. aureus	250
P. aeruginosa	125
P. mirabilis	500
E. faecalis	1000

Table 4: Phytochemical analysis of Sri Lankan variety *Cucumis melo* L. aqueous seeds extract

Secondary metabolites	Aqueous seed extract
Alkaloids	+
Diterpenes	-
Glycosides	-
Flavonoids	+
Phenols	+
Saponins	+
Tannins	+

4. Discussion

Present study investigated *in vitro* antibacterial activity of aqueous seeds extract of Sri Lankan variety *Cucumis melo* L. against five common urinary tract infective bacteria (*E. coli*, *S. aureus*, *P. aeruginosa*, *P. mirabilis* and *E. faecalis*) using standard Kirby-Bauer's disk diffusion method which is widely used in investigating antibacterial activity of both natural and synthetic products [15, 16]. To our knowledge, yet the antibacterial activity of aqueous seeds extract of Sri Lankan variety *C. melo* has not been evaluated. Several studies conducted in different countries have shown higher antibacterial activity associated with various organic solvents extracts of *C. melo* other than water [15, 17], but in the current study, aqueous extract was used, as water extract of herbal materials is mainly used in traditional and folk medicine. This difference in the potencies of extracts could be associated with the antibacterial activity possessed due to the greater solubility of active compounds of plant materials in the different organic solvents.

In the present study, antibacterial activity of aqueous seeds extract of *C. melo* against test organisms were evaluated by comparing with standard antibiotics, gentamicin and ampicillin [18, 19]. Statistical analysis revealed that antibacterial activities were equipotent to these antibiotics used which is a novel finding. The findings also showed a dose dependency (500 µg/ mL, 1000 µg/ mL and 2000 µg/ mL) which indicated that the effect was genuine, causal and specific.

The aqueous seed extract showed the highest susceptibility against *E. coli* followed by *S. aureus*, *P. mirabilis*, *P. aeruginosa* and *E. faecalis*. The highest activity against *E. coli* was a remarkable finding as it is the commonest bacteria that cause UTI among the populations of all age groups [16, 5, 19]. In addition, several studies have also provided evidence for the high resistance in *E. coli* to many synthetic antibiotics [20, 21]. Studies conducted in different countries have shown variations in antimicrobial potencies with different parts of *C. melo* plant. Similar study have found absence of antibacterial activity against *E. coli* [18] which is not in agreement with our results. The discrepancy could be due to the fact that they have utilized different solvent (hexane), different method (well diffusion assay) and different plant material (seed oil). In contrast, few studies have shown antibacterial activity against *E. coli* that consistent with the current study [22, 23]. However, mild to moderate activities were shown by these studies, compare to strong activity in the present study. The reason for the differences could be due to the use of different solvents, different extract concentrations and variations in environment conditions.

The findings of the current study revealed that gram negative bacteria *E. coli* (22.00±2.64) showed a greater activity when compared to gram positive *S. aureus* (19.60±2.08) and *E. faecalis* (14.60±0.57). Yet, similar study stated that gram positive bacteria were more susceptible than gram negative bacteria [24]. The contradictory results could be attributed to the different bacterial species and different variety of plant materials used in the *in vitro* studies.

In the current study, macro broth dilution method was used to determine MIC [15]. *E. faecalis* which showed a minimum ZOI had a maximum MIC value. Though *P. aeruginosa* showed a considerably low inhibition zone compared to *E. coli* it had a least MIC value. Similar MIC value was shown by *E. coli* and *P. mirabilis*. MIC values obtained in this study are low compared to MIC values of other similar studies [24, 25] which indicates that aqueous seeds extract of Sri Lankan variety *C. melo* possesses greater antibacterial activity. In addition, as yet there was no study conducted to determine MIC of aqueous seeds extract of Sri Lankan variety *C. melo*. Therefore, current study was the first investigation done to determine its MIC values against common uropathogens.

In the current study, phytochemical screening of the aqueous extract of *C. melo* seeds revealed the presence of alkaloids, flavonoids, phenols, saponins, tannins and the absence of diterpenes and glycosides. Metabolites like alkaloids provide defensive mechanism by intercalation with the cell wall and DNA of the microbes. Phenols cause disruption of the cell membrane while metabolites like flavonoids and tannins form complexes with bacterial cell wall, damaged cell membrane and cause enzyme inactivation [26], whereas saponins act as cell membrane permeabilizing agent [27]. Therefore, most likely mechanism for the antibacterial activity exhibited by the extract could be due to the synergistic action of these secondary metabolites via multiple actions [28]. In addition,

extract also possesses very remote chance to develop resistance against pathogens as it consists of mixture of complex secondary metabolites instead of a single compound. To our knowledge, present study was the first study to evaluate phytochemical screening of aqueous seeds extract of *C. melo* in Sri Lanka.

A preliminary phytochemical analysis in different countries have shown similar secondary metabolites, while some of their findings were contradictory to the present study [29, 30, 25]. The reason for the contradictory findings could be due to the utilization of different solvents, different variety of fruits and variations in environmental factors such as temperature, humidity, pH of the soil, soil fertility, irrigation and rainfall [11].

Environmental changes affect the quality and quantities of phytoconstituents in *C. melo* thereby affecting its effectiveness [11]. *C. melo* used in the current study was collected from a vegetable farm at Rajanganaya in dry zone of Sri Lanka. Its significant variation in antibacterial activity compared to other similar studies could be due to the variation in these secondary metabolites.

Use of *C. melo* extract by replacing synthetic antibiotics or in combination with antibiotics could reduce adverse effects as total exposure to antibiotic would be less. Traditional and folk medicine in Sri Lanka use seeds of *C. melo* for the treatment of infections associated with urinary tract [9]. Findings of the current investigation showed that seeds of *C. melo* possessed strong antibacterial activity against *E. coli, S. aureus, P. aeruginos, P. mirabilis* and *E. faecalis* that confirmed the great potential of bioactive compounds.

To our knowledge, no investigations have been conducted on aqueous seeds extract of Sri Lankan variety of *C. melo.* Accordingly, the current study was the first study that provided a novel finding for the Sri Lankan variety of *C. melo.* In addition, the results of the study scientifically justify the claim made by Sri Lankan traditional and folk medicine that seeds of Sri Lankan variety of *C. melo* is useful in the treatment of urinary tract associated infections. The results of the study also indicated the potential of aqueous extract of *C. melo* in developing a new drug that could be used to treat urinary tract infections.

5. Conclusion

In conclusion, this study, for the first time, showed that aqueous seeds extract of Sri Lankan variety *Cucumis melo* L. possessed an *in vitro* antibacterial activity against common urinary tract infective bacteria (*E. coli, S. aureus, P. aeruginosa, P. mirabilis* and *E. faecalis*). The highest activity was found against *E. coli* which is the most common uropathogen. Antibacterial activities were equipotent with standard antibiotics used which is a novel finding. The extract mediated antibacterial activity via synergistic mechanism of its secondary metabolites. The results of the current study, scientifically justified the claim made by Sri Lankan traditional and folk medicine. The findings also indicated the potential of developing a safer, cheaper and new drug for urinary tract infections.

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