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Non-flavonoid Polyphenol of *Allium cepa* Tuber Inhibits Bacterial Urease and the Proliferation of Lung Cancer Cells

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Abstract **Introduction:** Vegetables are excellent sources of bioactive compounds with multidimensional therapeutic potentials including UTIs. Uropathogenic bacteria *Proteus* plays a crucial role in the development of UTI or catheter-associated UTI. By decomposing the urea of human urine, it can cause catheter encrustation. **Objective:** This study investigated the antiureolytic and anticancer activities of fifteen vegetables and spinaches. **Materials and methods:** The enzyme urease was purified from uropathogen *Proteus mirabilis* by ion exchange column chromatography. Thirty aqueous and petroleum ether extracts from fifteen vegetables were tested to inhibit bacterial urease. **Results:** The extracts of *Allium cepa* tuber robustly inhibited urea hydrolyzing activity of urease followed by *Brassica oleracia* flower and *Lablab niger* fruit. The urease inhibitor compound designated as E8 in *Allium cepa* tuber was identified as a nonflavonoid polyphenol. The MIC of E8 for the inhibition of urease was estimated at 0.39µg/ml and the E8-mediated inhibition of urease was enzyme-specific, i.e., mechanism-based directed, not the active site-directed. E8 might have a high affinity to urease. The compound E8 also had anticancer activity against the lung cancer cell line A549 which predominantly stopped the cell division of A549 cells at the cytokinesis stage in a dose-dependent manner compared to the standard doxorubicin. The IC_{50} value of E8 for cancer cell proliferation was calculated at 160µg/ml from a regression line. **Conclusion:** The phenolic compound E8 of *Allium cepa* tuber can be a therapeutic agent for the target of lung cancer and kidney stone prevention.

Keywords: *Allium cepa tuber, polyphenol, antiureolytic, anticancer activity*

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1. Introduction

Medicinal plants contain bioactive chemicals which are the major sources of therapeutic agents to cure human diseases [1]. In respect of the widespread application of medicinal plants in numerous medical remedies, bioactive compounds have been expanded in the management of UTIs. Thirty-one species in Bangladesh are reported to be effective as traditional healers for UTIs [2-3]. The development of UTI is caused by various uropathogens, among them *Proteus* species are most predominant followed by *E. coli*, *Candida*, and *S. saphrophyticus*, etc. Decomposing organic and inorganic substances of human urine, *Proteus* bacterial species can contribute to catheter encrustation, cystitis, pyelonephritis, bacteremia, etc. Urease is only the pathogenic factor of *Proteus* bacteria responsible for this pathogenesis. About 10–15% of kidney stones are triggered by *Proteus* through the urease-dependent urine alkalization pathway, splitting urea into ammonia and carbon dioxide [4-5] and nearly 50% of urethral catheter indwelled patients for at least 5 days may develop bacteriuria or candiduria [6]. Therefore, *Proteus*-mediated infections show long-term complications causing tissue necrosis [7] and inflammation [8] at the site of infection as well as generating cytotoxic hemolysins [9]. While there are multiple treatment options available for managing kidney stones, there has been no discovery of natural sources that can effectively target and inhibit the

principal pathogenic factor, urease. Urease inhibitors have attracted much attention as potential antiulcer and anti-UTI drugs [10-11] and they have also been extensively examined by researchers [12-14] but the compound(s) as urease inhibitor(s) in *Allium cepa* tuber has not been studied. Identifying a natural inhibitor of urease could potentially overcome the risk factors associated with conventional medications and surgical therapies, providing an efficient and safer approach for kidney stone management.

Plant extracts have been found to possess potent anticancer properties due to the presence of bioactive compounds such as alkaloids, flavonoids, phenolic acids, terpenoids, and others. These compounds have been shown to interfere with various pathways involved in cancer development and progression, including cell cycle regulation, angiogenesis, apoptosis, and metastasis. Some plant extracts may also enhance the immune system's ability to recognize and eliminate cancer cells [15-16]. Polyphenols, bioactive peptides, polysaccharides, protease inhibitors, or protein hydrolysates exhibit a wide array of activities e.g., antiproliferative, apoptotic or cell cycle arrest, and inhibition of colon carcinogenesis [17]. Polyphenol-based drugs have been proven as potential therapeutic agents against various diseases. Fisetin (3,7,3′,4′-tetrahydroxyflavone) belonging to the polyphenolic subgroup flavonoids together with quercetin, myricetin, and kaempferol is found in several fruits and vegetables including strawberries, apples, persimmons, and onions. Fisetin, a highly useful natural agent, has been shown as a potent inhibitor of cancer in several *in vitro* and *in vivo* studies [18]. Tea polyphenols have anticancer anticarcinogenic or antimutagenic activity [19].

Many studies have investigated the potential anticancer properties of the extract of *Allium cepa* bulb. It contains various bioactive compounds, including flavonoids, phenolic acids, and organosulfur compounds, that have been linked to its antitumor effects [15]. These compounds have been found to induce apoptosis in cancer cells, inhibit cell proliferation, and block angiogenesis. Studies have also demonstrated that *Allium cepa* bulb extract has a protective effect against certain types of cancer including gastric cancer, breast cancer, colon cancer, and lung cancer [15]. However, some studies have reported that *Allium cepa* extract may enhance the efficacy of chemotherapy and reduce chemotherapy-induced toxicity [20]. However, a similar study of *Allium cepa* tuber, commonly used as a vegetable, has not been tested for anticancer potential.

Quercetin, a polyphenol extracted from onion skin, has been shown for its enzyme inhibitory effects on α-glucosidase and α-amylase that can delay carbohydrate digestion, thereby decreasing glucose absorption and reducing the postprandial plasma glucose elevation. Therefore, onion skin may be effective for the treatment of patients with type II diabetes mellitus [21]. Although the exact mechanism of *Allium cepa*-mediated anticancer properties is not fully understood, it is believed to involve the inhibition of cell cycle progression, induction of DNA damage and repair pathways, and modulation of signaling pathways involved in cancer cell survival and proliferation [15-16].

This study aimed to isolate and identify a bioactive molecule from *Allium cepa* tuber for the inhibition of urease, a predominating pathogenic factor of *Proteus mirabilis* responsible for UTI that will ultimately play a crucial role in reducing the severity of UTI and the formation of kidney stones. More precisely, this study aimed to reduce UTI-related complications as well as to explore bioactive molecule(s) for cancer management.

2. Methods and Materials

Chemicals and bacterial strain

All organic solvents were purchased from Active Fine Chemicals Limited, Dhaka, Bangladesh. The ion-exchange column resins (DEAE-cellulose and Q-sepharose FF from Sigma-Aldrich) were obtained as a generous gift from Prof. Toshinobu Tokumoto, Shizuoka University, Japan. *Proteus mirabilis*, a urease-positive bacterial strain was taken from lab stock which was isolated from a urine specimen of a catheterized inpatient. All other chemicals were of analytical grade.

Plant specimen collection and extraction

15 seasonal vegetable and spinach specimens were collected from November 2016 to February 2017 from the local market of Rajshahi, Bangladesh since the prevalence of UTI is more likely in winter. 100 g dried powder of each specimen was subjected to an extraction process by water and petroleum ether (Table 1) and 30 test extracts were obtained in this way. At a large-scale level, 4 kg dried power was obtained from 80 kg *Allium cepa* tuber and was extracted with petroleum ether. The solvent was evaporated to dryness by a rotary evaporator (Rotary Evaporator, RE 300, Bibby Sterilin Ltd, UK) at 50°C.

Column chromatography

A non-stick free-flowing mass of 15 g silica was prepared, loaded onto the column (46×2 cm^2), and equilibrated the column bed with hexane. The crude extract of *Allium cepa* tuber was loaded onto the equilibrated column and the column-bound chemicals were eluted with the solvent system comprising a different ratio of hexane and methanol from the lower (hexane) to the higher (methanol) polar region. A total of 322 eluted fractions were subjected to a test of TLC analysis to check the chemical components present in each fraction with a solvent system n-hexane: ethyl acetate (5:1) followed by the visualization under UV light, iodine chamber, and vanillin/$FeCl_3$/sulphuric acid spray. Elutes of closely similar bands were combined and evaporated to dryness, and by this way, ten fractions (F1 to F10) were obtained which were tested for antiureolytic activities (Figure 3C). The chemical components present in each combined fraction were analyzed on TLC under the solvent system n-hexane: ethyl acetate (5:1 and 10:1) (Figure 3D). In parallel, the fractions were tested for their antiureolytic activities, and the active fractions (F3, F4) were pooled, checked their banding pattern on TLC, combined, conducted further chromatography, bound chemicals were eluted again in ten fractions designated as E1 to E10 (Figure 4B). The active elute E8 was taken for the subsequent study.

Table 1. List of vegetables and spinach used

Local Name	English Name	Scientific Name	Family	Part used	Acronym
Piaj	Onion	*Allium cepa*	Liliaceae	Tuber	Ac-t
Deshi Seem	Hyacinth bean	*Lablab niger*	Fabaceae	Fruit	Ln-f
Fulcopy	Cauliflower	*Brassica oleracia*	Brassicaceae	Flower	Bo-f
Gajor	Carrot	*Daucus carota var. sativa*	Apeaceae	Root	Dc-r
Karola	Bitter gourd	*Momordica charantia*	Cucurbitaceae	Fruit	Mc-f
Misti Kumra	Pumpkin	*Cucurbita moschata*	Cucurbitaceae	Fruit	Cm-f
Patol	Pointed gourd	*Trichossanthes dioica*	Cucurbitaceae	Fruit	Td-f
Shosha	Cucumber	*Cucumis sativus*	Cucurbitaceae	Fruit	Cs-f
Begun	Brinjal	*Solanum melongena*	Solanaceae	Fruit	Sm-f
Barbati	Yard long bean	*Vigna sesquipedalis*	Fabaceae	Fruit	Vs-f
Lal Shack	Red amaranth	*Amaranthus gangeticus*	Amaranthaceae	Leaf	Ag-l
Palong shack	Spinach	*Spinacia oleracia*	Chenopodiaceae	Leaf	So-l
Dhonia pata	Coriander leaf	*Coriandrum sativum*	Apiaceae	Leaf	Sc-l
Mula	Radish	*Raphanus sativus*	Brassicaceae	Root	Rs-r
Tomato	Tomato	*Solanum lycopersicum*	Solanaceae	Fruit	Sl-f

15 specimens were selected based on the readily available and widely consumable Bangladeshi vegetables and spinach in the winter season since the prevalence of UTI is more likely in winter.

Bacterial culture and cell lysate preparation

From our lab stock, the uropathogenic bacteria *Proteus mirabilis* isolated from catheterized inpatients was taken, and cultured at 37°C in 1% urea-supplemented nutrient broth for 72 hr with mild shaking (60 rpm). 20g of bacterial cells were collected by centrifugation (2000 rpm, 10 minutes) and washing with 50mM PBS buffer (pH 7.4). The cells were crashed in lysis buffer (50 mM phosphate buffer pH 7.4, 50 mM NaCl, 1% Triton X-100, 1 mM EDTA, and 5% Glycerol) by high-speed vortexing for 10 min and repeated freeze-thaw cycle. The suspension of crushed cells was centrifuged at 10,000 rpm for 30 minutes. The supernatant was used for enzyme purification.

Purification of urease

The urease was partially purified following the methods described elsewhere [22]. The cell lysate (30ml) was loaded firstly on DEAE-52 sepharose and then Q-sepharose ion exchange columns equilibrated with buffer (50mM PBS pH 7.4, 50mM NaCl, 5% glycerol). The columns were washed with the same buffer and eluted with the elution buffer containing a concentration gradient of 50 mM to 1M NaCl. The urease-containing fractions from 125–250 mM for the DEAE-52 column and 200–225 mM for the Q-sepharose column were pooled. The OD_{280nm} and urease activity of each fraction were assayed for both column elutes. Then, elutes of the second column having high urease activity were used for further analysis.

Enzyme activity assay

10 ml of Christensen's broth (0.1% peptone, 0.1% glucose, 0.5% NaCl, 0.2% KH_2PO_4, 2% urea, and 0.0012% phenol red) adjusted to pH 6.5 was inoculated with 100µl of fresh bacterial suspension adjusted to OD_{650nm} at 0.5 (~10^8 cfu/ml) in a graduated culture tube and incubated at 37ºC undisturbed. Urea hydrolysis by urease produced by cells was started at the bottom towards the top of the culture tube. The time-dependent change of color of the culture broth from yellow to red was visualized and noted based on the values taken spectrophotometrically at OD_{595nm}.

Test of antiureolytic activity

The inhibition of urease activity was conducted by each of the column elutes of petroleum ether extract with purified urease. 20µl of urease solution and 100µl of column elute were mixed with 1380µl of reaction buffer (0.5% NaCl, 0.2% KH_2PO_4, 2% urea, and 0.0012% phenol red, pH 6.5) in an Eppendorf tube and incubated at 37ºC for 6 hr. The ability of each elute to inhibit ureolytic activity was determined colorimetrically by monitoring the pH rise caused by ammonia production in solutions containing urease samples with 40mM urea as described elsewhere [23]. The increase in absorbance of a pH indicator Phenyl Red at 557nm was used as a measure of the pH increase caused by ureolysis [24]. The unchanged color of the reaction mixer (yellow) indicated the urease inhibitory effect of elute.

Ex-vivo anticancer activity assay

Cytotoxic activity was carried out against human non-small-cell lung cancer (NSCLC) cell A549 using a slight modification of the Trypan Blue Exclusion Method [25-26]. Cells were cultivated in 75cm^2 flasks in 5% (v/v) CO_2 at 37°C with media described by Khan *et al* [27]. According to the study design, cells were grouped into 3 with three replicas each. Treatment groups were evaluated with the vehicle group. Cells were split on the day before experiments. The freshly prepared doses (5, 10, and 20 µg/mL) were administered 1 day before cultured T-flasks. Negative control corresponds to the cells cultured with media with 0.6% DMSO and Doxorubicin as a positive control. After 24 hr of incubation, cells were harvested using 0.5% trypsin. The number of dead cells was calculated by an automated cell counter (LUNA-II™, Analytikjena, South Korea) using trypan blue (0.4% w/v). The percentage of dead cells was calculated following the mathematical formula: Percentage of dead cells = (Number of stained dead cells / Total number of cells) ×100.

Statistical Analysis

Microsoft Excel 2013 was used for data analysis. Each value was obtained from triplicate experiments and the mean values were plotted.

3. Results

Inhibition of urea hydrolysis by vegetable extracts

Aqueous and organic (petroleum ether) extracts of

fifteen vegetables were screened for their antiureolylic activities on *P. mirabilis* urease. Four extracts out of thirty designated as Ac-t_a, Ac-t_o, Bo-f_a, and Ln-f_o were found to inhibit the hydrolysis of urea by urease (Figure 1). Both aqueous and organic extracts of *Allium cepa* tuber (Ac-t_a, Ac-t_o) exhibited inhibitory effects on urea hydrolysis in a qualitative test of analysis. In addition, the aqueous extract of *Brassica oleracia* (Bo-f_a) and the organic extract of *Lablab niger* (Ln-f_o) also showed antiureolytic activity (Figure 1).

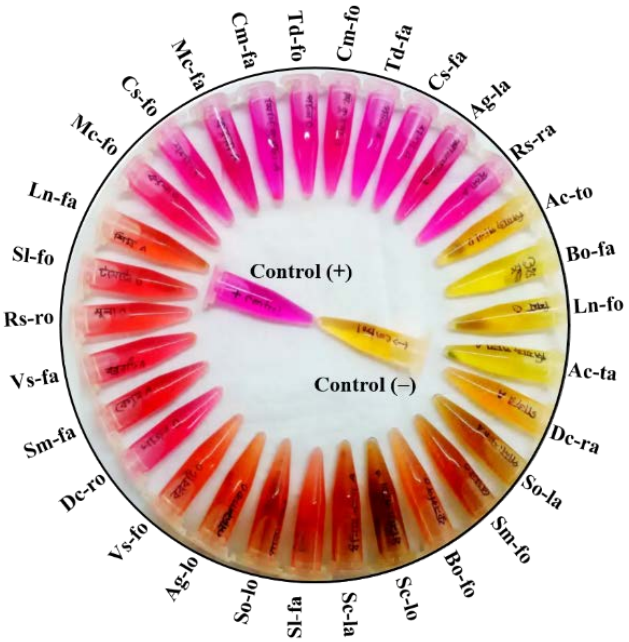

Figure 1. Test of urease inhibition by the vegetable extracts. The inhibition was found in samples treated with Ac-t_a, Ac-t_o, Ln-f_o, and Bo-f_a extracts since the color of the reaction mixture was unchanged as it was in the negative control

Table 2. Enzyme concentration-dependent inhibition assay

Scientific name	Solvent	Acronym	Extract (µl)	Result
Allium cepa	Organic	Ac-t_o	25	+
			50	+
	Aqueous	Ac-t_a	25	–
			50	–
Lablab niger	Organic	Ln-f_o	25	–
			50	–
Brassica oleracia	Aqueous	Bo-f_a	25	–
			50	–

Allium cepa tuber: a urease inhibitor

The extracts obtained from the above screening viz. Ac-t_a, Ac-t_o, Bo-f_a, and Ln-f_o were subjected to a follow-up enzyme inhibition assay at their low concentrations to check for their potentialities. Only Ac-t_o was found to inhibit the urea hydrolysis but others were not (Table 2, Figure 2B) which indicated that Ac-t_o might have one or more active compounds as urease inhibitor(s). Urease is a highly substrate-specific enzyme. Therefore, the same experiment was conducted using purified urease to confirm the inhibitory effect of Ac-t_o on urea hydrolysis. We found that Ac-t_o more robustly inhibited urea hydrolysis than the Ac-t_a (Table 2). The result strongly inferred that phytochemical(s) as urease inhibitor(s) was present in Ac-t_o.

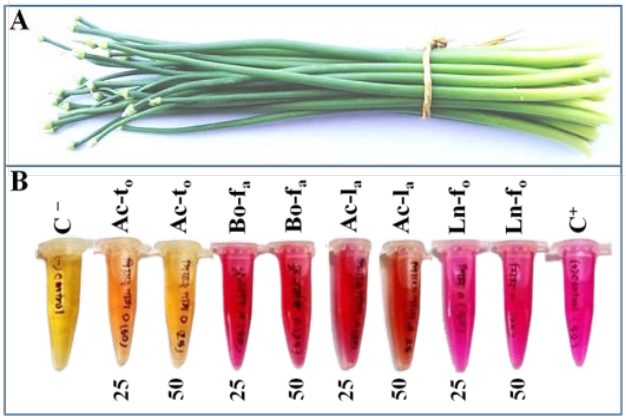

Figure 2. A: A bundle of *A. cepa* tuber. **B**: Test of urease inhibition by Ac-t_a, Ac-t_o, Ln-F_o, and Bo-f_a extract. The unchanged color of the reaction mixture for Ac-t_o extract indicated the inhibition of urea hydrolysis

Isolation of urease inhibitor

To isolate the target compound(s) from *Allium cepa* tuber the column chromatography with a large amount of organic extract was conducted as described in the materials and methods section. A total of 322 fractions were eluted and the compounds present in each elute were checked by their separation on TLC using the solvent system n-hexane: ethyl acetate (5:1) as mobile phase and examined under UV light, iodine (I_2) chamber, and vanillin/ sulphuric acid spray reagent (Figure 3). Elutes exhibiting similar chemical bands on TLC were combined and ten combined fractions obtained in this way were designated as F1 to F10. By carrying out the assay of urea hydrolysis with the combined fractions we assumed that F3, F4, and F10 might have the target compounds (Figure 3C) but by TLC analysis we decided to carry forward with F3 and F4 since their banding patterns were well separated and closely similar (Figure 3D). F10 was also carried over in parallel. After analyzing TLC, two different chemical constituents in different quantities were visualized in F3 and F4 (Figure 3D, arrowhead), therefore, the dose-dependent enzyme inhibition assay of both fractions was conducted separately to know their potentialities.

Table 3. Dose-dependent inhibition of urea hydrolysis by F3 and F4

Serial Dilution	Concentration (µg/L)	Result*	
		F3	F4
1	100.00	+	+
2	50.00	+	+
3	25.00	+	+
4	12.50	+	+
5	6.25	+	+
6	3.13	+	+
7	1.56	+	–
8	0.78	–	–

*Positive and negative signs indicated urea hydrolysis reaction and inhibition of reaction respectively. F3 was more potent (2-fold) than F4.

Dose-dependent enzyme inhibition

Minimum inhibitory concentrations (MICs) of F3 and F4 for urea hydrolysis were determined by serial dilution technique using 100 to 0.7813µg/L concentrations for each (Table 3). The MICs of F3 and F4 were estimated at 0.78 and 1.56µg/L respectively which indicated that F3 was two-fold stronger than F4. Thus, the TLC analysis led us to confirm that the components enclosed in the bracket (Figure 3D) were responsible for the inhibition of urea hydrolysis since these target components were higher in amount at F3. Both fractions were combined, subjected to the second column chromatography, and obtained elutes were screened for their effects on urea hydrolysis as described in the materials and methods section.

Identification of the chemical nature of the compound

The different tests for the identification of major phytochemicals present in F3 and F4 were carried out. They were primarily found as phenolic compounds (Figure 4A, lane 1,2). No terpenoids, alkaloids, or flavonoids were detected by separate tests (data not shown). F_c, the combined fractions of F3 and F4, was loaded on the second column, eluted with the solvent system, and elutes (E1 to E10) were tested for the inhibition assay of urea hydrolysis. Only elute E8 was able to inhibit urea hydrolysis (Figure 4B). The purity of compound E8 obtained was checked out which was about 70–80% pure and further detected as a phenolic compound (Figure 4A, lane 3,5).

MIC determination

MIC of E8 for the inhibition of urea hydrolysis was determined by the serial dilution technique as described earlier. The MIC was estimated at 0.39µg/ml (Figure 5) which was two-fold stronger than F3 (Table 3). It indicated that the phenolic compound present in E8 had a high binding affinity to enzyme or substrate or enzyme-substrate complex and thereby was responsible for the inhibition of urea hydrolysis.

Effect of E8 on pH of reaction mixer

The compound E8 was tested for its effect on the reaction pH whether or not the inhibition of urea hydrolysis by the addition of E8 was a consequence of a drastic change in pH (6.50) of the reaction buffer. But the addition of E8 had no significant effect on the reaction pH (6.45) indicating that the inhibition of urea hydrolytic reaction occurred by either enzyme- or substrate-specificity to E8.

Mechanism of urease inhibition

We further expanded our assumption in quest of how the phenolic compound, E8, inhibited urea hydrolysis by urease. To chase our question E8 was assessed to find out whether the inhibition was substrate- or enzyme-specific. Two possibilities were taken into consideration, i.e., (1) E8 interacted with the substrate to hinder its binding to the active site of the enzyme, (2) the enzyme itself was modulated and thereby inactivated by the binding of E8. Both enzyme and substrate were pre-treated with E8 separately and the inhibition assay was conducted colorimetrically following the method described above. At the end of the incubation period, the unchanged color of the reaction mixer for the E8 pre-treated enzyme solution indicated that the E8-mediated inhibition of urea hydrolysis was enzyme-specific (Figure 6). One possible explanation might be the 3D structural modulation of urease by E8 during the pre-treatment reaction.

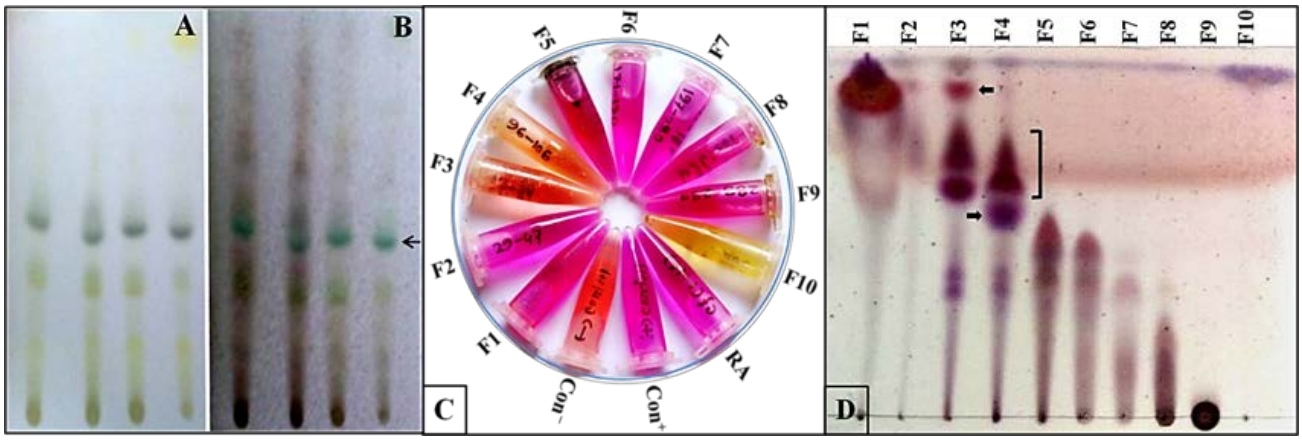

Figure 3. Chromatographic separation of crude extract. The solvent system of n-hexane and ethyl acetate (5:1) was chosen for chromatography. The separated bands on the TLC plate were visualized under UV (**A**) and $FeCl_3$ spray (**B**). A total of 322 fractions were eluted. Each fraction was subjected to TLC analysis to check the banding pattern of the compounds. Fractions having almost similar bands were combined. Ten combined fractions (F1 to F10) obtained were tested for their urease inhibitory effect. F3, F4, and F10 were found to be the same as negative control (Con^-) in colorimetric analysis (**C**). **D**: The banding pattern of phytochemicals present in combined fractions on a TLC plate. Fractions (F3, F4) shared four common bands, two of them were more intensified (bracket) and differed in two separate bands (arrowheads). The common bands enclosed by the bracket might be the targets that are responsible for urease inhibition

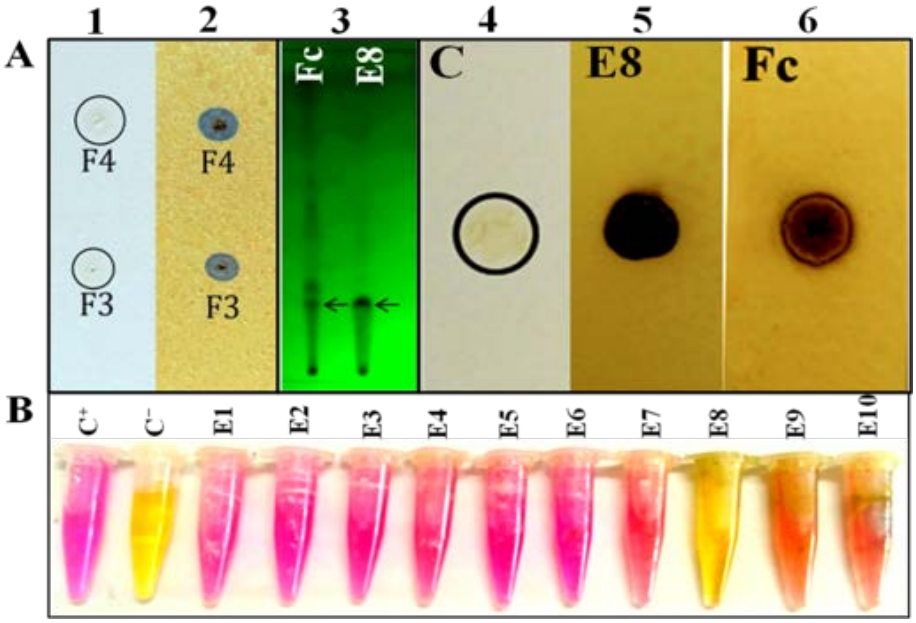

Figure 4. Identification of the chemical nature of the compound in F3 and F4. $FeCl_3$ solution was sprayed over the spotted fraction on the TLC plate and turned its color into black indicating the presence of the phenolic compound in fractions (**A**, lane 2). The purity of E8 was checked and compared to the combined fraction Fc (**A**, lane 3). The fraction E8 was about 70–80% pure with slight impurities at the baseline. **A**: Spotted compound before spray (lane 1), sprayed with $FeCl_3$-ethanol solution (lane 2). **B**: Further separation of combined fractions of F3 and F4. Eluted fraction E8 showed urease inhibition. **C**: sprayed with Dragendorff's reagent (lane 4), Fc: Combined fractions sprayed with $FeCl_3$ (lane 6), E8: Pure compound (E8) sprayed with $FeCl_3$ (lane 5)

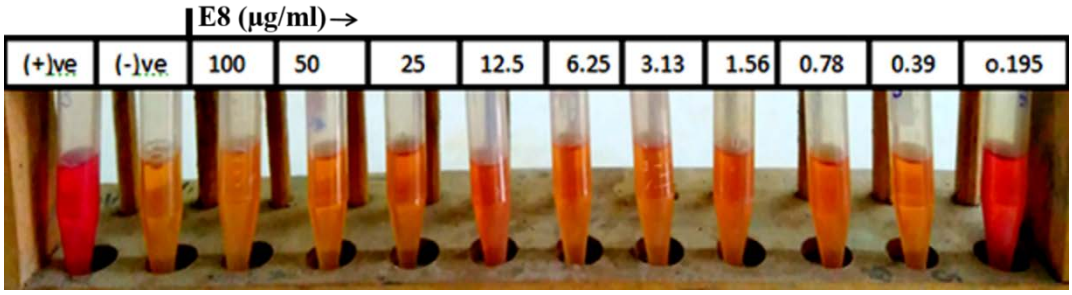

Figure 5. MIC determination. The urea hydrolyzing reaction was conducted in the presence of variable concentrations of E8 and the change of color of the reaction mixture was compared with positive and negative control. The MIC of E8 was found to be 0.39µg/ml

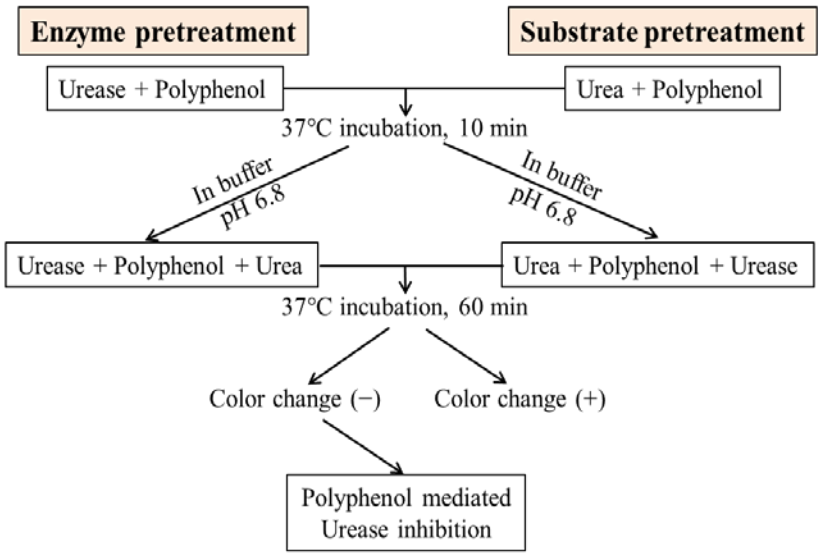

Figure 6. Mechanism of polyphenol-mediated inhibition of urease activity. The enzyme pre-treated with phenolic compound at 37°C for 10 min could not raise the reaction pH and no change in the color of the reaction mixer was found

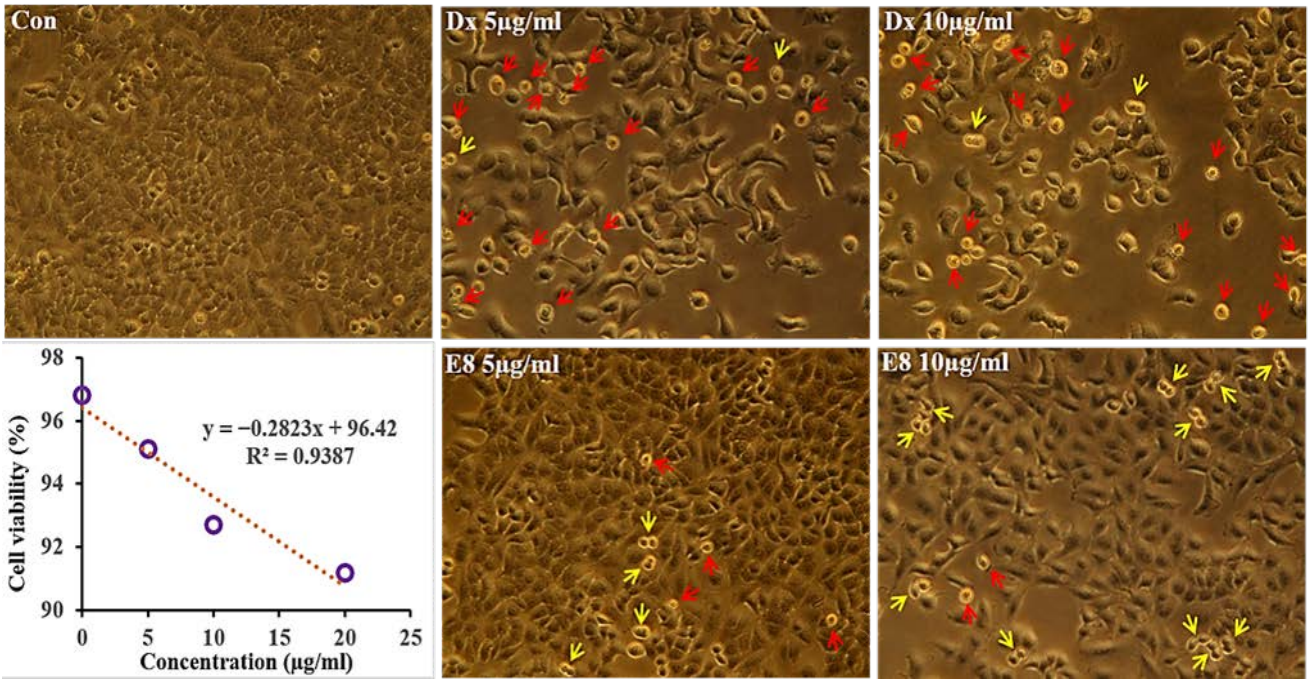

Figure 7. Morphological observation of apoptosis of human lung cancer cell line A549 treated with doxorubicin (Dx) and polyphenol (E8) after 1 day of treatment. Red arrowheads indicate apoptotic cell death and yellow arrowheads indicate cell cycle arrest at G2/M metaphase. The percentage of cell viability of apoptotic A549 cells was estimated at 1, 5, 10, 20 µg/ml concentration of E8. The calculated IC_{50} was found to be 160µg/ml

Effect of E8 on A549 cancer cell viability

Phenolic compounds isolated from medicinal plants have been considered interesting agents for their chemopreventive and chemotherapeutic effects on cancer cells for a long time. These phytochemicals are classified into several subgroups viz. simple phenols, lignans, phenylpropanoids, flavonoids, coumarins, etc [28]. Therefore, to assess the effect of E8 on cell viability of lung cancer cell A549 was carried out. The reference standard doxorubicin (Dx) showed the induction of cell apoptosis in a concentration-dependent manner whereas E8 was found predominantly to stop the cell division during the cell proliferation phase in a concentration-dependent manner. Compared with the control there was a constant decrease in the cell viability of increased concentration of E8 treatment (Figure 7). The IC_{50} value of E8 was calculated at 160µg/ml from a regression line.

4. Discussion

Many studies have been reported on the *Allium cepa* bulb which is commonly used as a spice in our daily life but the *Allium cepa* tuber, a winter vegetable, has rarely been studied. Phenolic compounds of *Allium cepa* bulb have potential antiinflammatory, anticholesterol, anticancer, and antioxidant activities [18]. In this study, we isolated a phenolic compound from *Allium cepa* tuber that exhibited antiurease activity *in vitro* and anticancer activity *ex vivo*. Phenolic compounds are the dominant antioxidant components in medicinal herbs. Major types of this compound include phenolic acids, flavonoids, tannins, coumarins, lignans, quinones, stilbenes, and curcuminoids which exhibit far stronger antioxidant activity [19]. Catechin, a flavonoid belonging to the subgroup of polyphenols extracted from green tea, has been shown as a strong inhibitor of *Helicobacter pylori* urease with an IC_{50} value of 13 µg/ml [29]. Our isolated phenolic compound E8 was a non-flavonoid type (MIC 0.39 µg/ml) that was stronger than catechin. Traditional medicinal plants containing anticancer activities are good sources of potent natural antioxidants as well as chemopreventive agents [19]. Phenolic compounds derived from germinated legumes exert a wide range of beneficial effects and the most relevant is anticancer activity [17]. Although the phenolic compounds usually contribute a lot to their antioxidant activities sometimes they do not necessarily possess good cytotoxic activities on cancer cells [30] but the phenolic compound derived from *Allium cepa* tuber in this study possessed cytotoxic activity in the lung cancer cell line (Figure 7).

Urease inhibitors act as effective therapeutic agents for the treatment of diseases caused by urease-dependent pathogenic microorganisms. However, the commercially available urease inhibitors, e.g., phosphorodiamidates, hydroxamic acid derivatives, and imidazoles are toxic and less stable which restricts their clinical uses. Plant-derived polyphenols have been reported with remarkable inhibitory effects on the ureases of *Helicobacter pylori*, *Canavalia ensiformis*, and soil microbiota [30]. The urease inhibitions were extensively studied because of their potential uses like– (i) therapy against bacterial urease that induced human pathogenic states, such as urinary stone formation, peptic ulcer, pyelonephritis, and hepatic coma [31], (ii) to protect soil from pH elevation and loss of nitrogen after use of urea fertilizer by controlling hydrolysis of urea in soil [32], and (iii) as an analytical technique for determining substances acting as enzyme inhibitor [33]. In this study, we showed polyphenol of *Allium cepa* tuber mediated inhibition of urease, a dominating virulence factor of uropathogenic bacteria *Proteus mirabilis*, responsible for UTI and catheter-associated UTI. Eleven EtOH and five MeOH extracts of herbs screened by Ghous *et al*. showed that

herb extracts of *Achillea millefolium* and *Aristolachina bracteata* had strong inhibitory effects against the ureolytic activity of *Helicobacter pylori* urease with IC_{50} values of 12 and 11.75 µg/ml respectively [34]. On the contrary, we found the MIC value of E8, a vegetable polyphenol, at around 0.39 µg/ml against the ureolytic activity of *P. mirabilis* urease (Figure 5) which was much stronger than that of herbs. In another study, thirty commercially available compounds out of seventy-one were found to inhibit the ureolytic activity of *Klebsiella pneumoniae* cells and of purified jack bean urease where two phenolic compounds– tannic acid, and gallic acid were noted as urease inhibitors. Tannic acid and gallic acid inhibited 100% and >23% of the ureolytic activity of purified jack bean urease respectively [35]. Another article reported the inhibition of urease by MeOH extract of *Allium ascalonicum* leaf on five *Helicobacter pylori* cells in a concentration-dependent manner but no MIC or IC_{50} values were detected by them [36]. Plant juices obtained from *Allium sativum* (garlic), *Allium cepa* (onion), *Allium porrum* (leek), and *Brassica oleraceae* (cabbage or sprouts) were shown to be effective urease inhibitors [37]. We also found the antiureolytic activity of the aqueous extract of *Brassica oleraceae* (Bo-f_a) which was less effective than that of *Allium cepa* tuber (Figure 1, Table 2). Urease inhibition by *Brassica* juice was completely reversible but that by *Allium* juice was 50% reversible [37]. In this contest, *Allium cepa* is preferable to *Brassica oleraceae*. Our present study could not determine whether the E8-mediated urease inhibition was reversible or irreversible. Urease inhibitors are broadly classified into two categories– (i) active-site directed (substrate-like), and (ii) mechanism-based directed (non-substrate-like) [10]. We assumed that E8-mediated urease inhibition was mechanism-based directed and the urease molecule might have the high affinity binding site(s) for E8.

Recently 1,918,030 new cancer cases and 609,360 cancer deaths are found to occur in the United States where around 350 deaths per day from lung cancer, a leading cause of cancer death [38]. Fruit, vegetables, and some beverages (tea, coffee) are particularly rich in dietary polyphenols that may exert their anticancer effects through several possible mechanisms, such as the removal of carcinogenic agents, the modulation of cancer cell signaling and antioxidant enzymatic activities, and the induction of apoptosis as well as cell cycle arrest [39]. In the present study, we assessed the anticancer property of E8 against the lung cancer cell line A549 and obtained E8-induced inhibition of cell proliferation (Figure 7). The IC_{50} value of phenolic compound E8 was estimated at 160 µg/ml. The cytotoxic effect of EtOAc and dichloromethane extract of *Dillenia suffruticosa* root enriched in polyphenolic compounds on the A549 cancer cell line was shown by Armania *et al*. with an IC_{50} value of 35 µg/ml which was due to the induction of apoptosis and cell cycle arrest at G2/M phase of cell proliferation [40]. Based on the IC_{50} values, E8 was 4.5 times weaker than the dichloromethane extract of *Dillenia suffruticosa*. Dieckol a phenolic compound isolated from brown algae *Ecklonia cava* inhibits lung cancer cell A549 proliferation. It has been shown as a potent anticancer agent with an IC_{50} value of 25µg/ml [38]. The compound E8 was 6.5 times weaker than dieckol. In this study, we observed that A549 cell proliferation was stopped at the cytokinesis stage during the E8-treated cell division (Figure 7) which might be the cell cycle arrest at the G2/M phase although we could not do the flow cytometric analysis due to the lack of an instrument. Resveratrol, a well-known plant-derived polyphenol was tested on a panel of lung cancer cell lines by Maj *et al*. and it showed antiproliferative activity against all the lung cancer cell lines but with different potency [41]. Moderate activity was observed against the A549 cell line with an IC_{50} value of 40–60µM [42]. Similarly, E8 derived from *Allium cepa* tuber might have different potentialities for different cancer cell lines although it was weaker than some well-known polyphenols. We cannot rule out the possibility that other test vegetables except *Allium cepa* tuber do not possess any component(s) having antiureolytic or anticancer activities. Only we demonstrated here that *Allium cepa* tuber had more potent urease inhibitors than others concerning the inhibition of urea hydrolysis.

5. Conclusion

This study has substantiated that plant extracts can serve as a valuable source of urease inhibitors and components for cancer. Fifteen market-available vegetable and spinach species have undergone the screening process and among them, *Allium cepa* tuber has emerged as a successful candidate in these investigations. The polyphenolic compound of *Allium cepa* showed a robust inhibition of the urease of *Proteus* bacteria, so it can be a therapeutic agent for the target of kidney stone prevention. Moreover, it has also demonstrated its potential as an anticancer agent, particularly in combating lung cancer cells. Further investigation is strongly encouraged to uncover the structural composition and molecular arrangements of the compounds exhibiting these potentials.

Authors' Contribution

S and AS carried out the experimental works and S drafted the rough manuscript; MNU partly helped in the experimental work; MAR, MRA, IAS, MBY, MHL, and MRS supported the study with data collection and handling; AUC conceptualized the study, analyzed the data, and finalized the manuscript.

Conflict of Interest

Authors declare no conflict of interest.

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