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Antibacterial Activity of *Cissus quadrangularis* Stem Extract on the Pathogenic and Industrial Waste Watered Bacteria

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ABSTRACT

Cissus quadrangularis (Vitaceae) is a popular climber conspicuous by its flesh quadrangular stem widespread throughout the Bangladesh. The *in vitro* antimicrobial activity of *C. quadrangularis* extracts was studied against selected pathogenic bacteria, industrial wasted bacteria, and broth dilution assay. The most commonly used method of microbiological assay is the disc diffusion method. *C. quadrangularis* stem extracted with four solvents (Petroleum spirit, methanol, ethyl acetate, and dichloromethane) were tested for antimicrobial activities against some pathogenic microorganisms *Sarcina lutea* (002-1), *Xanthomonas campestris* (004-1), *Escherichia coli* (005-1), *Klebsiella pneumonia* (006-1) and some industrial (Tannery, Tobacco, and Sugar mill) waste watered bacteria by disc diffusion method. Among the four extracts, ethyl acetate showed moderate antibacterial activity against *X. campestris* (004-1) and industrial watered bacteria. But, the commercial disc Oxicycline doesn't show any antibacterial activity against the industrial waste watered bacteria. Petroleum spirit, methanol, and dichloromethane extract were ineffective against all of the tested bacteria.

Keywords: *Cissus quadrangularis,* Antibacterial effect, Industrial waste, Stem extract, Pathogenic, and Tannery.

INTRODUCTION

Medicinal Plants have been used as a source of medicine since the dawn of civilization. The plant designed as medicinal is implied that it is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Various medicinal plants have been applied for years in daily life to treat disease all over the world (Nair *et al*., 2004). The stem contains two unsymmetrical tetracyclic triterpenoids, and two steroidal principles. The presence of β-sitosterol, δ-amyrin, δ-amyrone, and flavonoids (quercetin) having different potential metabolic and physiological effects have also been reported (Jainu and Devi, 2004). The ulcer protective effect of a methanolic extract of *C. quadrangularis* was similar to that of the reference medicine sucralfate (Jainu and Devi, 2004). Many imitate agents such as estrogens in hormone replacement therapy, especially estrogen receptor modulators have been designed to treat osteoporosis but each one of them is linked with side effects such

as hypercalcemia, hypercalciurea, raised risk of endometrial, and breast cancer, breast tenderness, menstruation, thromboembolic events, vaginal bleeding and hot flushes (Ogey *et al*., 2001; and Sanyal and Ahmad, 2005).

C. quadrangularis (Linn) has been applied by common people in Bangladesh for promotion of fracture healing and well known as Hadjod. It is also known as *Vitis quadrangularis* wall which belongs to family Vitaceae. Some other news on *C. quadrangularis* noticed its effectiveness in management of obesity, and symptoms linked with metabolic disorders (Oben *et al*., 2006), as well as its antioxidant and free radical scavenging pursuit *in vitro* (Mallika and Shyamala, 2005).

Phytochemical studies of *C. quadrangularis* have shown the presence of different versatile components such as flavanoids, triterpenoids, Vitamin C, stilbene derivatives and many others, e.g. resveratrol, piceatannol, pallidol perthenocissin, and phytosterols. Out of which ascorbic acid, triterpene, β-sitosterol, ketosteroid, two asymmetrical tetracyclic triterpenoids and calcium were identified as major constituents of this plant (Jainu and Devi, 2004; and Enechi *et al*., 2003). The root powder also contain a rich source of mineral elements (mg/100g dry matter): potassium 67.5, calcium 39.5, zinc 3.0, sodium 22.5, iron 7.5, lead 3.5, cadmium 0.25, copper 0.5 and magnesium 1.15 (Somova *et al*., 2003). Fresh stems of *C. quadrangularis* develops irritating action on the skin, which may be attributed to the existence of calcium oxalate and 31 methyl tritiacontanoic acid along with taraxeryl acetate, taraxerol and isopentacosanoic acid (Prajapati *et al*., 2003).

Bangladesh is based with various kinds of plant and most of them have medicinal properties. The ash formed from the *C. quadrangularis* contains mostly carbonates and to a smaller extent phosphates of sodium, potassium, magnesium and calcium. Presence of potassium tartarate is also reported (Austin *et al.,* 2004). The extract measured well with Acetyl salicylic acid (Viswanatha *et al*., 2006). As it measured well with acetyl salicylic acid its analgesic responses with the nature of its chemically active components needs to be explored (Jainu and Devi, 2004). The ethyl acetate fraction of both fresh and dry stem extracts at a concentration of 100 ppm showed 64.8% antioxidant activity in the β-carotene linoleic acid system and 61.6% in the 1, 1-diphenyl-2- picrylhydrazyl systems (Furukawa *et al*., 2004). It works by preventing the transformation of arachidonic acid to inflammatory prostaglandins (Mallika and Shyamala, 2006).

The optimum preservative dose of 500 mg/kg of extract was applied for the pretreatment of gastric ulcers with various doses of CQE (250, 500, and 750 mg/kg) for 7 days which significantly attenuated these biochemical alters caused by aspirin in rats (Sanchez-Fidalgo *et al*., 2004). So, the plant has been taken into consideration with a keen interest to investigate the following aims and objectives: Isolation of the extract from *C. quadrangularis* stems using different solvents such as ethyl acetate, dichloro methane, and methanol and petroleum spirit; observation of the antibacterial activity of different extract of *C. quadrangularis* against various infectious bacteria and industrial waste watered bacteria; comparisonal study of commercial discs and prepared discs of *C. quadrangularis* stem on bacterial growth; and determination the MIC values of using different solvent extracts of *C. quadrangularis* stem against different bacterial strain and industrial waste watered microbes.

MATERIALS AND METHODS

The experiment has been conducted in Microbiology and Biochemistry laboratory at Gono University, Savar, Dhaka, Bangladesh for the screening of antimicrobial activity, is a typical microbiological assay, which is performed with culture of microorganisms. The zone of inhibition is compared in millimeter (mm) unit. This is a measure of antibacterial activity of the test compound. The materials and methods applied in this investigation are described below under the following heading:

Plant Material: *Cissus quadrangularis* stem.

Collection of Plant Material: The *C. quadrangularis* stem was collected from Dhaka District of Bangladesh in the month of September, 2019. There is usually a wide choice among liquids to be applied as solvents for extraction operation. However the following

solvents were applied in this experiment: Methanol, Ethyl acetate, Dichloro methane, and Petroleum spirit.

Collection of Industrial Waste Water: Three (3) samples of waste water were collected from the following sources - Tannery waste water from Hazaribagh; Tobacco waste water from leaf factory, Gazipur; and Sugar mill waste water from Faridpur sugar mill, Faridpur.

Culture of Waste Watered Bacteria: Two types of media were applied, viz., MacConkey Agar Media and Nutrient Agar media. Each of the waste water was cultured to MacConkey agar media and Nutrient agar media was applied for spread culture. For stick culture, the Nutrient agar media was applied. Then some of the colonies were transferred to conical flask for liquid culture with the help of Nutrient broth.

Bacterial Species: Gram negative i.e. *E. coli* (005-1), *K. pneumoniae* (006-1) and Gram positive i.e. *S. lutea* (002-1) and *X. campestris* (004-1) were applied in the present study to determine the antibacterial activity of the different extract.

Culture of Bacterial Species: For culture of bacterial species from the stock culture of bacteria, lactose broth media was applied. For preparation of lactose media, suspended 0.325 gm of powdered broth in 25 ml distilled water in a conical (100ml) flask. Then the each species of stock cultured bacteria were transferred to each conical flask in front of laminar air flow. Then the flasks were shaked in a shaker machine about 24 hrs. The major approaches for testing the antimicrobial activities of extracts were disc diffusion method, agar dilution method (Luangtongkum *et al*., 2007) and the broth dilution method (Kianbakht and Jahaniani, 2003). In this experiment the disc diffusion method was applied.

Fig 1: Preparation of stem sample and fine powder.

Extraction Methods: After collection of the *C. quadrangularis* stem were cleaned with rinsed water and cut down the stem and then air dried and then it was pulverized into a fine powder (**Fig 1**).

Extraction from the Powder Sample: Ten (10) gm of the *C. quadrangularis* stem powder were weighted separately with electric balance and 60 ml each of the solvent (methanol, ethyl acetate, dichloromethane, and petroleum spirit) were added in each conical flask. The powder was dissolved separately with methanol, ethyl acetate, and dichloromethane and petroleum spirit. The samples with solvent were placed in water bath shaker for 24 hours at (30-36) ^{o}C for proper extraction.

Extraction of the Extract: The extract of plant materials was filtered. This was performed by passing the extracts through Whatman filter paper.

Concentrating the Extract and Preparation of Disc: The extracts were then air dried after filtration to concentrate. The filter paper was punched with the punching machine and the disc was made. The discs were taken into a Petri dish and sterilized in an autoclave for 15 minutes with 121 ºC and 15 psi pressure.

Bacterial Culture Media: For cultivation and maintenance of different bacterial culture and for the identification and microbial sensitivity, nutrient agar was applied. Nutrient agar medium; a microbiological culture medium commonly applied for the routine cultivation of non-fastidious bacteria. Also, it can grow on the surface of the Nutrient agar and is clearly visible as tiny colonies. In nutrient broth, the bacteria grow in the liquid, and were visible as a soupy component, not as clearly distinguishable clumps.

Preparation of Media for the Tested Organisms: In this study, nutrient agar media was applied for antibacterial screening. For the test, 5.6gm of nutrient agar was dissolved into 200ml distilled water in 250ml conical flask. The media was properly dissolved with the distilled water and then sterilized in an autoclave for 15 minutes with 121 ºC and 15 psi pressure. After autoclaving, the media was poured into the autoclaved Petri dishes in the laminar air flow cabinet.

Table 1: Composition of the culture media was used.

Composition of Lactose Broth (LB) Media		**Composition of Nutrient Agar (NA) media**	
Ingredients	Amount(gm/l)	Ingredients	Amounts(gm/l)
Peptone	5.00	Peptic digest of the animal tissue.	5.00
Meat (beef)	3.00	Sodium chloride	1.50
Lactose	5.00	Beef extract	1.50
		Yeast extract	1.50
		Agar	15
pH (at 25 ^{o}C) 6.9±2		pH (at 25 ^{o}C) 6.8 ± 0.2	

Minimum Inhibitory Concentrations (MIC) Determination of *C. quadrangularis* Stem Extracts: In the experiment, Minimum inhibitory concentrations (MICs); as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. MICs were applied by diagnostic laboratories prominently to confirm resistance, but most often as a study tool to examine the *in vitro* activity of new antimicrobials, and data from such studies have been applied to evaluate MIC breakpoints. The method gives information on the depot of standard antibiotic powder, preparation of stock antibiotic solutions, media, and production of inocula, incubation periods, and reading and interpretation of findings. In the present study, it was determined following the serial dilution. The lowest concentrations of the extracts, which did not show any growth of tested organisms after microscopic evaluation, were determined as MIC (Rahman *et al*., 2019).

Sample Solution Preparation: Stock working solution of the plant stem extract was prepared by dissolving 0.316 gm dried stem extracts in 10 ml solvent (ethyl acetate) into a separate flask. So it was to be 10 times diluted. Then 10 ml of ethyl acetate solvent was added in the same flasks. Then 162.00 µl extract solution was transferred into a screw capped test tube and 838.00 µl of same solvent was added in the same test tube. Therefore, the final concentration was reached to 512 µg/ml.

Serial Dilution: For preparing 512 µg/ml to 2 µg/ml, 1ml of the solvent was added to each of the nine screws capped test tube. 1 ml of the having 512 µg/ml extracts was added to the 1st test tube containing 1ml of particular solvent and mixed well in the vortex and then 1ml of this solvent was shifted to the second test tube containing 1ml of the same solvent. After mixing well, 1ml of this mixture was shifted to the third test tube. This process of serial dilution was continued up to the last test tube. Finally, the clustered of the last test tube was 2 µg/ml.

Preparation of Working Disc for Antimicrobial Test: The disc paper was soaked with each concentration of extracts and placed at room temperature for air dry for 15 hours. After completion of air dry, the disc paper was labeled according to different concentration and finally the labeled disc paper was taken into the vial and it was ready for antibacterial activity and the waste watered (Tannery, Tobacco, Sugar mill) bacteria.

RESULT AND OBSERVATION

Determination of Antibacterial Activity of Ethyl Acetate Extract of *C. quadrangularis* Stem: From the **Table 2** it has been shown that the stem powder of the *C. quadrangularis* showed greatest antibacterial activity against tested bacteria, viz., *X. campestris* (004-1). The crude extract of stem powder produced 12 mm zone of inhibition against *X. campestris* (004-1). It produced no zone of inhibition against other tested bacterial strain.

The ethyl acetate extracts of *C. quadrangularis* stem showed inhibitory activity against *X. campestris* (004-1) with 12 mm inhibitory zone. Commercial antibiotic disc (Penicillin) was applied as a positive control that showed anti-bacterial activity against *X. campestris* (004-1) (**Fig 2**).

Table 2: Diameter of the zone of inhibition produced by ethyl acetate extracts of *C. quadrangularis* against different bacterial strain.

Name and number of bacterial strain	Zone of inhibition (mm)		
	Commercial antibiotic disc	Extract of Ethyl acetate	Negative Control
***S. lutea* (002-1)**	30 (Kanamycin)	-	-
***X. campestris* (004-1)**	17 (Penicillin)	+	-
***E. coli* (005-1)**	17 (Ampicillin)	-	-
***K. pneumonia* (006-1)**	34 (Ciprofloxacin)	-	-

N.B. (-) Not Inhibition, (+) Inhibition

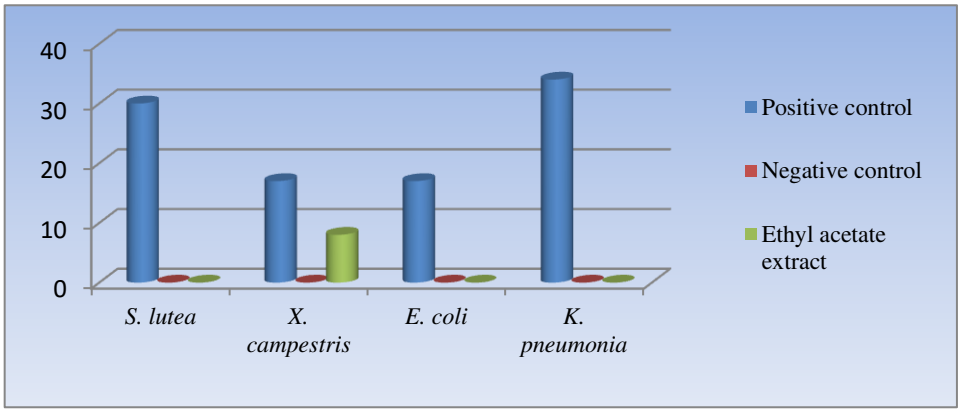

Fig 2: Comparative antibacterial activity of commercial disc, ethyl acetate extract of *C. quadrangularis* and ethyl acetate solvent against selected micro-organisms.

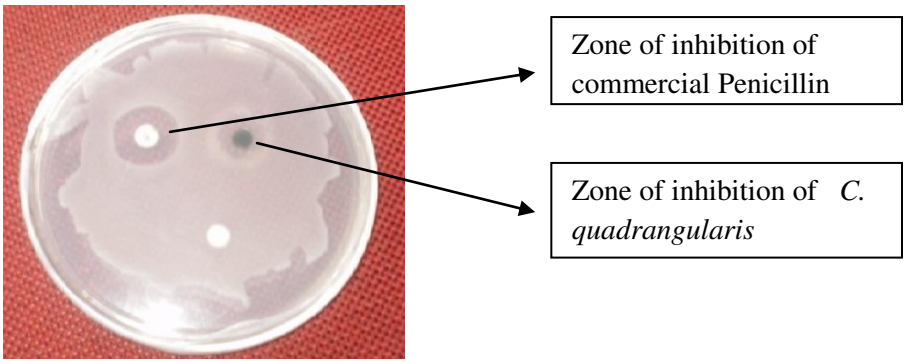

Fig 3: Zone of inhibition with ethyl acetate extracts of *C. quadrangularis* against *X. campestris* (004-1).

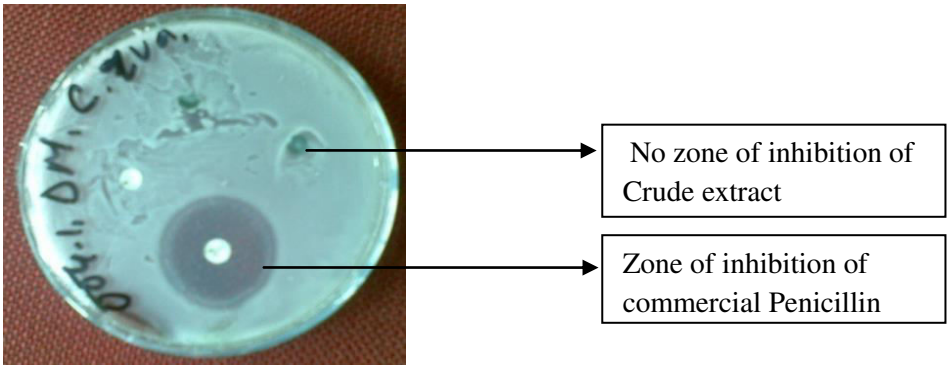

Fig 4: No zones of inhibition with dichloromethane extract of *C. quadrangularis* against *X. campestris* (004-1).

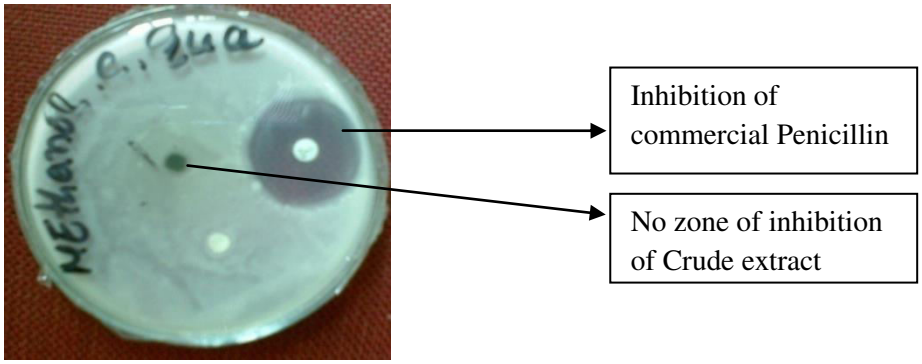

Fig 5: No Zone of inhibition with methanol extracts of *C. quadrangularis* against *X. campestris* (004-1).

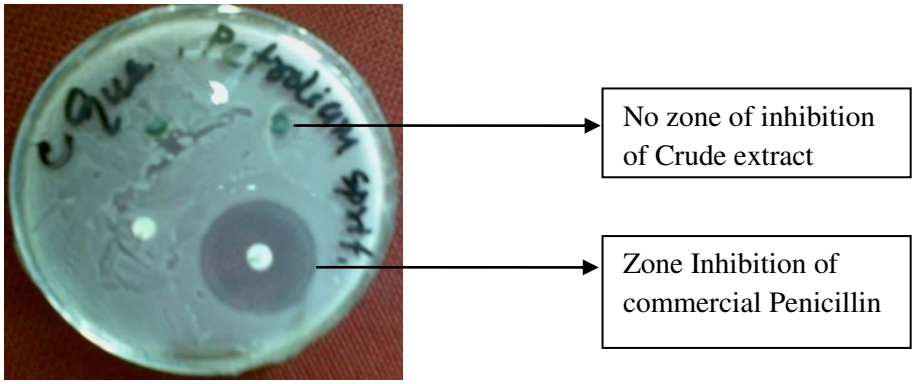

Fig 6: No zones of inhibition with petroleum spirit extract of *C. quadrangularis* against *X. campestris* (004-1).

Table 3: MIC of Ethyl Acetate against *X. campestris* (004-1)

Microorganisms	**Concentration of Ethyl acetate extract (µg/ml)**							
	1024	512	256	128	64	32	16	Control
S. lutea	-	-	-	-	-	-	-	-
X. campestris	+	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-	-
K. pneumoniae	-	-	-	-	-	-	-	-

N.B. (-) Not Inhibition, (+) Inhibition

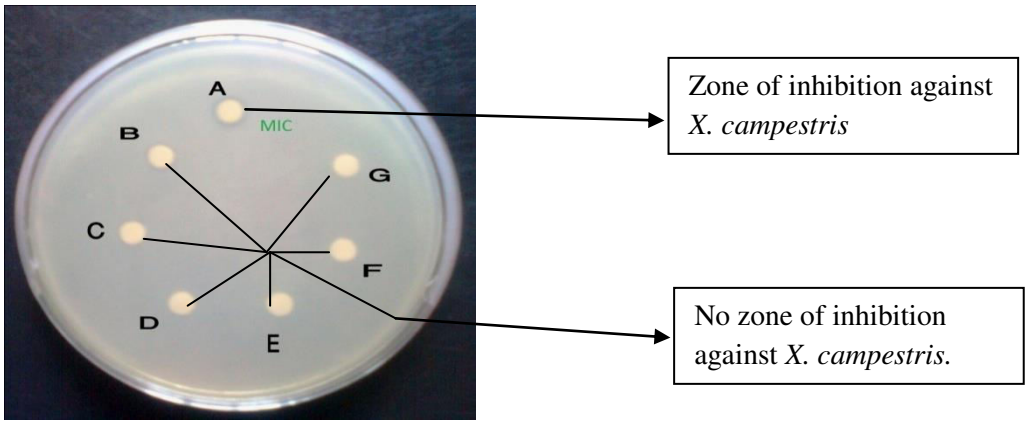

Fig 7: MIC of ethyl acetate extract against *X. campestris* (004-1); A, B, C, D, E, F, G disc contain 1024, 512, 256, 128, 64, 32, 16 µg/ml respectively.

The ethyl acetate extract shows an inhibitory zone at 1024 µg/ml concentration and does not show any antibacterial activity at the concentration of 512 µg/ml, 256 µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, and 16 µg/ml against *X. campestris.* So; the MIC value of ethyl acetate extract is 1024 µg/ml (**Table 3**).

Determination of Antibacterial Activity against Waste Watered Bacteria (Tannery, Tobacco, and Sugar Mill)

From the **Table 3** it has been shown that the stem powder of the *C. quadrangularis* showed antibacterial activity against bacteria that lived in waste water. The crude extract of stem powder produced 18 mm zone of inhibition against Tobacco waste water, 14 mm zone of inhibition against Tannery waste water and 16mm zone of inhibition against Sugar mill waste watered bacteria.

Table 4: Diameter of the zone of inhibition produced by ethyl acetate extracts of *C. quadrangularis* again different industrial waste watered bacteria.

Industrial Sample of Microbes	Diameter of zone of inhibition (mm)		
	Commercial antibiotic disc (Oxicycline)	Crude extract (mm)	Negative control
Tobacco	-	+	-
Tannery	-	+	-
Sugar	-	+	-

N.B. (-) Not Inhibition, (+) Inhibition

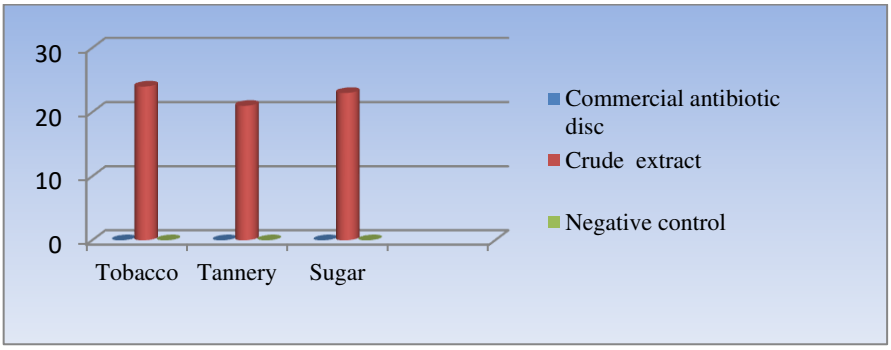

Fig 8: Comparative antibacterial activity of commercial disc, Ethyl acetate extract of *C. quadrangularis* and Ethyl acetate solvent against selected waste watered microorganisms.

Zone of inhibition of with ethyl acetate (EA) extract of *C. quadrangularis* stem against Tannery, Tobacco and Sugar mill waste water:

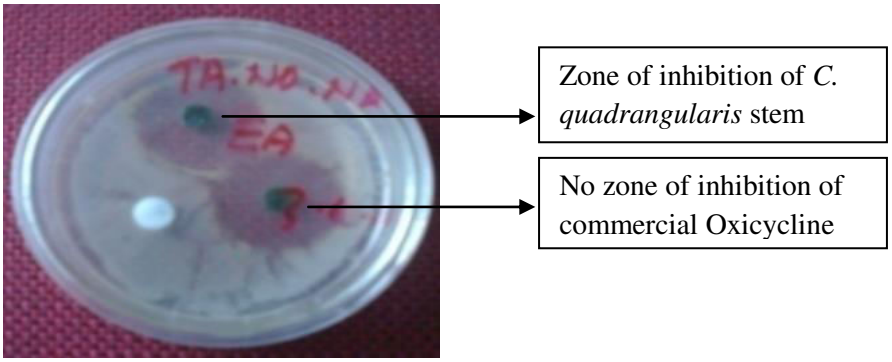

Fig 9: Zone of inhibition with ethyl acetate (EA) extract of *C. quadrangularis* stem against Tannery waste water.

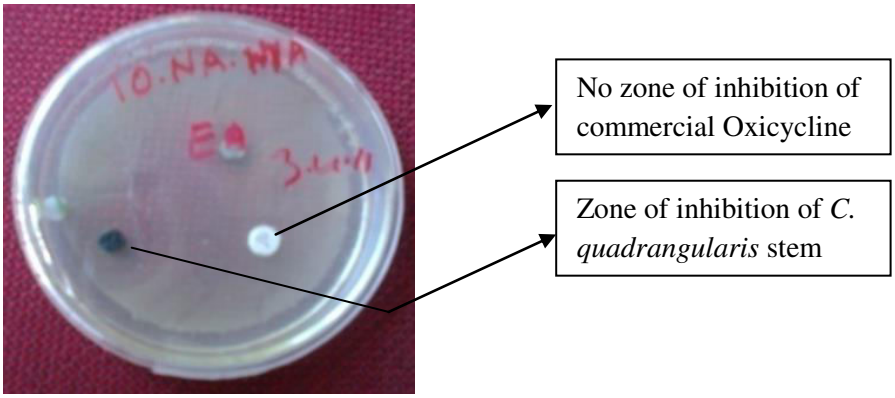

Fig 10: Zone of inhibition with ethyl acetate (EA) extract of *C. quadrangularis* stem against tobacco waste water.

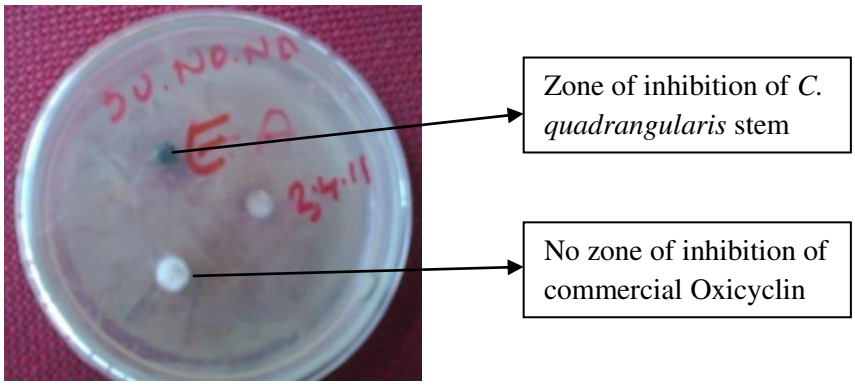

Fig11: Zone of inhibition with ethyl acetate (EA) extract of *C. quadrangularis* stem against Sugar mill waste water.

Table 5: MIC of Ethyl Acetate against Tobacco, Tannery and Sugar.

Industrial sources of microorganisms	**Concentration of Ethyl acetate extract (µg/ml)**							
	1024	512	256	128	64	32	16	Control
Tobacco	+	-	-	-	-	-	-	-
Tannery	+	-	-	-	-	-		-
Sugar	+	-	-	-	-	-	-	-

N.B. (-) Not Inhibition, (+) Inhibition

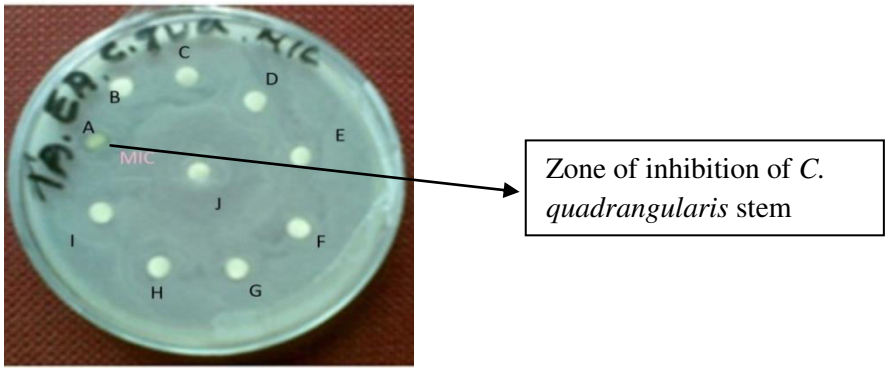

Fig 12: Zone of inhibition with ethyl acetate (EA) extract at A in 1024 µg/ml of *C. quadrangularis* stem against Tannery waste water.

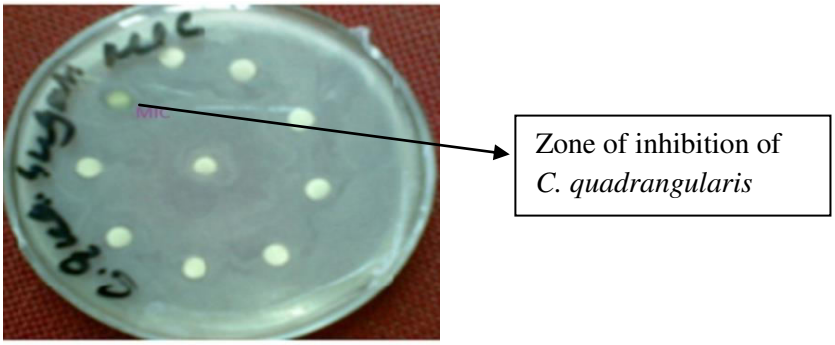

Fig13: Zone of inhibition with ethyl acetate (EA) extract at 512 µg/ml of *C. quadrangularis* stem against sugar mill waste water.

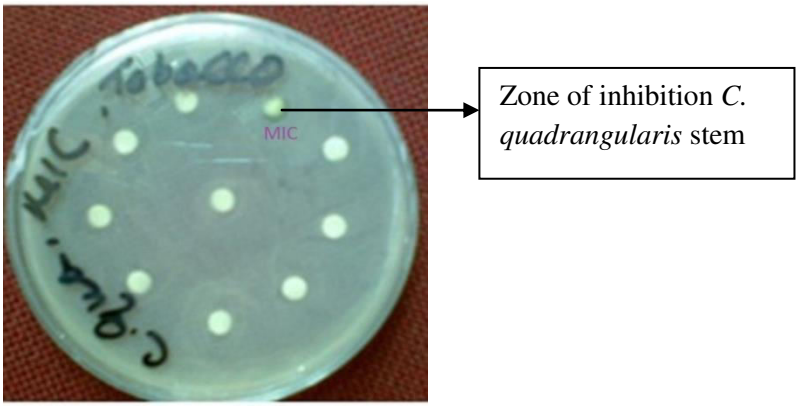

Fig 14: Zone of inhibition with ethyl acetate (EA) extract at 512 µg/ml of *C. quadrangularis* stem against Tobacco waste water.

The ethyl acetate extract showed antibacterial activity at the concentration of 512 µg/ml against all of the waste watered bacteria. But ethyl acetate extract did not show antibacterial activity at the concentration of (256 µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml, and 2 µg/ml) against all of the waste watered bacteria. Commercial antibiotic disc (Oxicycline) was applied as a positive control that did not show any anti-bacterial activity against waste watered bacteria.

DISCUSSION

Plants have supplied a major source of inspiration for novel drug components as plants derived drugs have made pivotal contribution towards human health. Phytomedicine can be applied for the therapy of diseases as is done in case of Unani and Ayurvedic system of drugs or it can be the base for the maturing of a medicine. The results of the antibacterial activity of stem of *C. quadrangularis* against the investigated bacterial strains and industrial sample of microbes were shown in the **Table 2** and **Table 4**. The present study proved the traditional use of *C. quadrangularis* as an antihemorrhoidal drug in Thai folk medicine (Murthy *et al*., 2003).

The increasing social and economic implication caused by pathogenic bacteria means there is constant striving to develop new antibacterial agents (Uddin *et al*., 2014). Due to the identified and potential toxicity of chemical antibiotics, there has been an increasing demand for safe and effective antibacterial from natural sources (Happy *et al*., 2018). Thus plants extracts are promising natural antibacterial agents with potential applications in pharmaceutical industries for controlling the pathogenic bacteria. In this experiment, isolation and characterization of some clinical enteric bacteria from laboratory of Gono University of Dhaka and industrial waste water sample from different industries and examined the effectiveness of a medicinal plants water bacteria (Sharif *et al*., 2019). The crude ethyl acetate extract of C. *quadrangularis* showed potentate antibacterial activity against *X. campestris* (004-1) strain in compared to commercial antibiotic disc Penicillin and antibacterial activity against some industrial waste water in compared to

commercial antibiotic disc Oxicycline. The presence of antibacterial substances in the higher plants is well established (Srinivasan *et al.*, 2001). It also produced 18 mm zone inhibition against Tobacco waste water, 14 mm zone of inhibition against tannery, 16 mm zone of inhibition against sugar mill waste water. So it has been observed that ethyl acetate extract of *C. quadrangularis* showed moderate antibacterial activity against pathogenic bacteria and industrial waste watered bacteria.

CONCLUSION

From the observed result of the research work it can be concluded that the *C. quadrangularis* stem extract inhibit the growth of pathogenic bacteria strain, possesses the potent antibacterial activity against pathogenic bacteria strain, antibacterial effect against industrial (tannery, tobacco, and sugar mill) waste watered bacteria, and can be applied as a therapeutic agent. The ethyl acetate, dichloromethane, methanol and petroleum spirit extract of *C. quadrangularis* was tested for their antibacterial activity against selected pathogenic bacteria (Firoz *et al*., 2016). The sample solution is applied on the test plate containing microorganism. The sample solution diffuses in the surrounding medium and the plates are kept in an incubator (37 ^{o}C) for 24 hours. The plant extract possess any antibacterial activity, it will inhibit bacterial growth in the surrounding area giving a clear zone of inhibition (Shahen *et al*., 2019). It can be concluded that the free radical scavenging activity of the plant extract may be in charge of for the treating action against tissue destroy (Gabriel *et al*., 2005). The following works can be performed in future: characterization of the active components of *C. quadrangularis* and further characterization of the microbes that present in industrial waste water.

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CONFLICT OF INTEREST

All the authors of this manuscript agreed that they have no conflict of interest to publish the manuscript.

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