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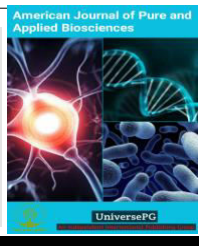

Determination of Antimicrobial Activity of Medicinal Plant *Cassia obtusifolia* L. (Chakunda) Leaf Extract on Selected Pathogenic Microbes

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ABSTRACT

Pathogenic microorganisms are major health concern of infectious diseases. In the present study ethanolic and methanolic extracts of *Cassia obtusifolia* leaves from Kushtia region (Bangladesh) were subjected to evaluate the *in vitro* microbial activity against six important human pathogenic bacteria viz., *Bacillus subtilis* (001-1), *Sarcina lutea* (002-1), *Xanthomonas campestris* (004-1), *Escherichia coli* (005-1), *Klebsiella pneumonia* (006-1) *and Pseudomonas sp.* (010-1) employing disc diffusion method. The crude methanolic extract of *C. obtusifolia* produced maximum area of inhibition (14 mm) against *S. lutea* (002-1) and crude ethanolic extract of *C. obtusifolia* produced largest area of inhibition (11 mm) against *K. pneumonia* (006-1). The MIC values (256 µg/ml, 512 µg/ml) were obtained from the methanolic isolate and ethanolic extract that produced 4 mm and 3 mm area of inhibition against *S. lutea* (002-1) and *K. pneumonia* (006-1). Methanol extract showed the greater activity than ethanol extract. The most susceptible bacterial strains to ethanol and methanol extracts were *S. lutea* (002-1) and *K. pneumonia* (006-1). So it may be possible that the production of new antibiotic from *C. obtusifolia* L. leaf may be recommended for meningitis and pneumonia. The findings of this research suggest that the extracts of *C. obtusifolia* L. can be a source of natural antibacterial agents with pivotal applications in pharmaceutical companies to control pathogenic bacteria causing severe illness in humans.

Keywords: *Cassia obtusifolia,* Antibacterial effect, Pathogenic bacteria, Leaf extract, and Disease management

INTRODUCTION

Plants are the rich source of bioactive elements and thus supply as necessary raw materials for drug production. They may constitute a valuable natural asset of a country and contribute a great deal to its health care system. Nearly 80% population of the world depends upon the traditional process of health care. Medicinal plants are mainly used for the treatment of different illness in India, as these are regarded to have applications over the traditionally applied drugs that are expensive and familiar to have injurious side effects (Khare and Verly, 2004). Aliphatic drugs have brought a revolution overall the world but the plant-based medicines have their own unique status (Behera *et al.,* 2006; Shahen *et al.*, 2019).

Decoctions of parts of *C. obtusifolia* are usage as an analgesic, anticonvulsant, antipyretic, anti-insecticidal antifungal, antihelmint, diuretic, expectoran, laxatif, purgatif, treatment of glaucoma and high blood pressure, treatment of scabies, ringworm and itch, etc. This plants has been described for its functionally in the type of decoctions, immersion and tinctures in common process of drugs for treating scabies like psoriasis, leprosy, etc (Cordova *et al*., 2002; and Harrison & Dorothy, 2003). Surveys have revealed that 50 percent of the top prescription drugs in the USA are based on natural products and the raw materials are locked up in the tropical world interiors of Africa, Asia, and America. The regional uses of harbs as a cure are usual particularly in those zones, which have tiny or no access to contemporary health system (Behera, 2006). Various levels of bioactive elements have been recognized from *C. obtusifolia* seed, such as anthraquinones, alkaloids, terpenoid, flavonoid, and lipid, etc (Liu *et al*., 2014).

C. obtusifolia is a little annual plant or undershrub growing as a popular weed in Asian states. It is found around subcontinent mostly. It is an annual fetid herb, 30–90 cm high. The leaves, bark and seeds are functional in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, heart diseases (Chan *et al*., 2001). *S. aureus* was current in more than 50% of patients with AD and PS, and establish that the seriousness of AD and PS necessarily connected to enterotoxin production of the extracted *S. aureus* strains (Tomi *et al*., 2005). Furthermore, Ju Ming Jiang made from this plant in union with chrysanthemum has a definite curative impact on hypertension, and sickle senna seed decoction, syrup and tablets are essential for hyperlipemia, the presence of huge fat or lipids in the blood stream (Tadhani and Subhash, 2006).

According to Edwards (2001), habitat and species are being lost rapidly as a result of the merged effects of environmental degradation, agricultural expansion, and deforestation and over-harvesting of species. *C. obtusifolia* extract has been focused to have an anti-Helicobacter pylori consequence, inhibitory effects on the extension of *Clostridium perfringens* and *E. coli*, estrogenic consequences, and inhibitory the consequences on histamine secrete from mast cells, and platelet gathering (Zhang *et al*., 2015).

The Govt. of Bangladesh officially identified Unani and Ayurvedic systems of medicine. The Arabians Muslim physicians like Al-Raze and Ibn Sina brought about a revolution in the history of medicines by bringing new drugs of plant minerals origin into general use. More than 250 of such medicinal plants are now common use in the preparation of traditional medicine in Bangladesh (Habib *et al.*, 2019). Predict the treatment benefits with the antibacterial agents analyzed, and suggest clinicians in selecting the very suitable agent for a distinct clinical disorder (Turnidge and Jorgensen, 2003). A research also focused that *C. obtusifolia* can diminished memory destruction in mice convince by scopolamine regime or transient bilateral popular carotid artery blockage and that these upshot were regulated via acetyl cholinesterase inhibition (Kim *et al.*, 2007). Study of the nutritional quality of *C. obtusifolia* leaves and kawal revealed that fermentation in the kawal processing has resulted in the increase of in vitro protein digestibility, fat, protein and ash content and a decrease in fiber content (Algadi and Yousif, 2015).

Antibacterial, anti-platelet aggregation, hepato-protective, cAMP phosphor-diesterase inhibitory activity, antifungal, anti-yeast, anti-inflammatory and antiestrogenic, Hypolimpidemic, anti-mutagenic and antioxidant activities of this herb has been evaluated (Duke *et al*., 2002; Karaman *et al*., 2003). *C. obtusifolia* is popularly used as a medicinal plant for the remedy of headache, dizziness, food borne diseases, and eye disease (Kim *et al*., 2011; and Sob *et al*., 2010). Bangladesh is based with various kinds of plant and most of them have medicinal properties (Khatun *et al.*, 2016). *C. obtusifolia* is the medicinal plant of Bangladesh as well as all over the world. The main objectives of the study is to isolate the extract from *C. obtusifolia* leaf using different solvents such as methanol, ethanol, and observe the antimicrobial activity of various isolates of *C. obtusifolia* against various infectious bacteria.

MATERIALS AND METHODS:

Antimicrobial activity screening is a classic microbiological assay, which is accomplished with the culture of microorganisms. The most ordinary used approach of microbiological assay is the disk diffusion method; the sample solution is applied to the test place containing microorganisms. The sample solution diffuses in the surrounding medium. Then the plates are kept in a hot air woven (37ºC) for 24 hours. If the plant extract possess any antimicrobial activity, it will inhibit microbial growth in the surrounding medium giving a clear area of inhibition. The area of inhibition is measured in millimeter unit. This is determining of antimicrobial activity of the test compounds. The methanol and ethanol extract of leaf of the *C. obtusifolia* were tested for their antimicrobial activity against a number of a pathogenic organisms.

Plant Samples and Chemical (Solvent) Used - *C. obtusifolia* leaf. The leaf was collected from various places of Islamic University, Kushtia Campus in the month of September to February. There is usually a wide choice among liquids to be used as a solvent for extraction.

Microbial Species and Nutrient Media – Gram-negative i.e. *E. coli* (005-1), *Pseudomonas sp* (010-1), *K. pneumoniae* (006-1) and gram-positive i.e. *B. subtilis* (001-1) *S. lutea* (002-1) and *X. campestris* (004-1) were used in the current research to examine the antimicrobial activity of the extract. For cultivation and maintenance of different bacterial culture and for the identification and microbial sensitivity, nutrient agar is used.

Nutrient agar is mainly used for the regular cultivation of non-fastidious microbes and it is beneficial because it rest solid even at comparatively high temperatures. Also, bacteria culture in nutrient agar medium grows on the surface and is obviously visible as little colonies. LB media was applied for culturing of the microbes. LB is also used for the isolation of coliform bacteria in water, milk products, and other type of materials.

Table 1: Composition of the various culture mediums with the constituents.

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**	

Instruments, Apparatus and Extraction of Leaf - Conical flask, Autoclaving bottles, Separating funnels, Filter paper, Glassol, Autoclave, Shaker, Laminar airflow, Incubator, Petri dishes, and Micropipette. After collection of the leaf was cleaned and then dried. Then it was pulverized into a fine powder.

Isolation and Extraction from the Powder Sample - 100gm of the Cassia leaf powder was weighted with electric balance and 300 ml of the solvent (methanol and ethanol) was added in each conical flask. The powder was extracted separately with methanol and ethanol. The samples with solvent were placed in a water bath shaker for 24 hours at 30-36 ^{o}C.

Filtration and Concentrating the Extract - The extracts of plant material i.e. methanolic isolate and ethanolic was filtered. This was performed by passing the extracts through filter paper. The extracts were then air-dried after filtration to concentrate.

Media and Disk Preparation - The filter paper was thump with the thumping machine, and the disk was made. The discs were taken into a Petri dish and sterilize in an autoclave machine for 15-20 minutes

with 121ºC temperature and 15 lbs-inch^2 pressure. In this study, nutrient agar (NA) medium was used for antibacterial screening. For the test, 5.60 gm of NA was dissolved into 200 ml distilled water in 250 ml conical flask. After autoclaving, the media was cooled for some time and poured into the autoclaved Petri dishes in the laminar airflow cabinet.

Estimation of Minimum Inhibitory Concentration (MIC) of *C. obtusifolia* Leaf Extract - MICs will inhibit the able to be seen growth of microbes after overnight incubation. MICs are used by diagnostic centres mainly to establish resistance, but most often as a study tool to examine the *in vitro* activity of new antibacterial and data from such research have been applied to analyze MIC breakpoints. This approach gives information on retain of good quality antibiotic grind, manufacturing of stock antibiotic solutions, media, and manufacturing of inoculums, incubation environment, reading, and elucidation of findings. The lowest concentrations of the extracts, which did not represent any growth of analyzed organisms after microscopic evaluation, were determined as MIC.

Sample Solution Preparation - Stock working solution of the plant leaf extract was prepared by dissolving 1.80 gm dried leaf extracts in 10ml solvent (methanol and ethanol) into a flask. So, it was to be 10 times diluted. Then 10 ml of methanol and ethanol solvent was added in the separate flasks. Then 284.44 µl extract solution was transferred into a screw-capped test tube and 715.56 µl of the same solvent was added in the same test tube. Therefore, the final concentration was reached to 512 µg/ml.

Serial Dilution of the Solvent - For preparing 512 µg/ml to 2 µg/ml, 1ml of the solvent was added to each of the nine screws capped test tube and 1 ml of the having 512 µg/ml extracts were added to the first test tube holding 1 ml of respective solvent and mixed well in the vortex and then 1 ml of this solvent was transferred to the second test tube holding 1 ml of the same solvent. After mixing well, 1 ml of this mixture was transferred to the third test tube. This approach of serial dilution was picked up to the last test tube. Finally, the concentration of the last test tube was 2 µg/ml.

Disc Preparation - The disc paper was immerged with each concentration of isolates and kept at room temperature for air dry for 15 hours and then dried disc paper was kept in the oven for 1 hour at 37˚C. After completion of oven-dry, the disc paper was marked as stated by to different concentration and finally, the marked disc paper was taken into the vial and it was ready for antimicrobial activity.

Determination of *In vitro* Antimicrobial Activity - Six organisms were analyzed this research to the determination of the antibacterial effect of crude extract. In antibacterial screening, nutrient agar was used as culture media. Then in vitro antimicrobial activities of the extracts were measured by employing the standard agar disc diffusion method. In the disc diffusion process, the disc was put down aseptically over the microbial culture on NA plates and incubated at 37˚C for 24 hours. After incubation for 24 hours, the area of inhibition around the disc was measured accurately by a millimeter scale. The Disc was impregnated with each treatment and control was assayed on duplicate agar medium plate for *B. subtilis* (001-1), *S. lutea* (002-1), *X. campestris* (004-1), *E. coli* (005-1), *K. pneumoniae* (006-1), and *Pseudomonas sp* (010-1). The experiment was replicated two times to confirm the reproducible results. Sterile blank disc was impregnated with sterile solvent (methanol and ethanol) and applied as negative control each test. Quality Nalidixic Acid (Na-30 µg/µl) was used as a positive control for contrast of the antimicrobial activity.

RESULT AND OBSERVATION:

Antimicrobial Activity of Methanolic Extract of *C. obtusifolia* Leaf - From the **Table 2** it has been shown that the leaf powder of the *C. obtusifolia* showed antimicrobial activity against some the bacterial strain as well as *B. subtilis* (001-1) *S. lutea* (002-1), and *E. coli* (005-1). The crude extract of leaf powder produced 14mm area of inhibition against *S. lutea* (002-1). It also produced an area of inhibition of 11 mm against *E. coli* (005-1) and 10mm area of inhibition against *B. subtilis* (001-1). The methanolic isolates of leaf showed the highest inhibitory activity against *S. lutea* (14 mm) compare to the other

organism tested. The crude methanolic isolate of leaf was not sensitive to *X. campestris* (004-1), *K. pneumonia* (006-1) and *Pseudomonas sp*. (010-1). Negative control (disc containing only the methanol solvent) exhibited no zone against the six (6) organisms tested. Commercial antibiotic disc, Nalidixic Acid (30 µg/µl) was used as a positive control that showed antimicrobial activity against six tested bacteria (**Fig 2**).

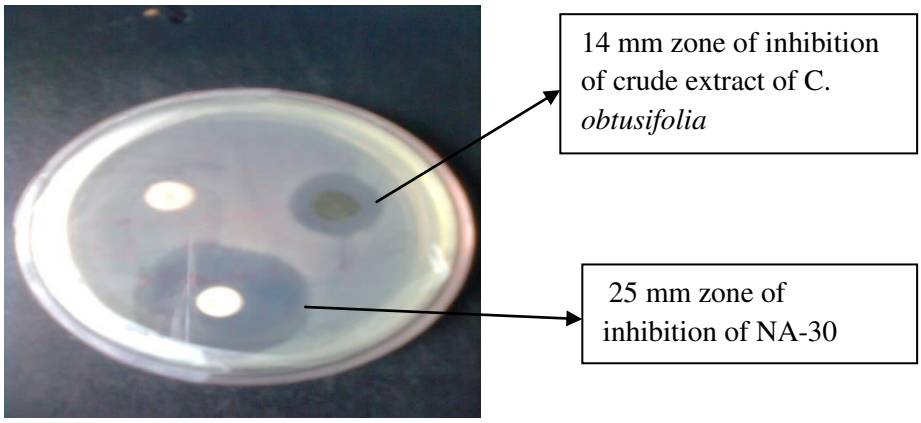

Fig 1: Zone of inhibition with methanol extract of *C. obtusifolia* leaf against *S. lutea* (002-1).

Table 2: Zone of inhibition produced by methanol extracts of *C. obtusifolia* against different bacterial strain.

Name of bacterial strain	Diameter of the zone of inhibition(mm)										
	Commercial antibiotic disc (Na-30) (30 µg/µl)	Crude extract	Extract concentration (µg/ml)								
			512	256	128	64	32	16	8	4	2	O
***B. subtilis* (001-1)**	26	10	-	-	-	-	-	-	-	-	-	-
***S. lutea* (002-1)**	25	14	6	4	-	-	-	-	-	-	-	-
***X. campestris* (004-1)**	12	-	-	-	-	-	-	-	-	-	-	-
***E. coli* (005-1)**	26	11	5	-	-	-	-	-	-	-	-	-
***K. pneumonia* (006-1)**	17	-	-	-	-	-	-	-	-	-	-	-
***Pseudomonas sp* (010-1)**	-	-	-	-	-	-	-	-	-	-	-	-

N.B. (-) No Inhibition, O - Negative control.

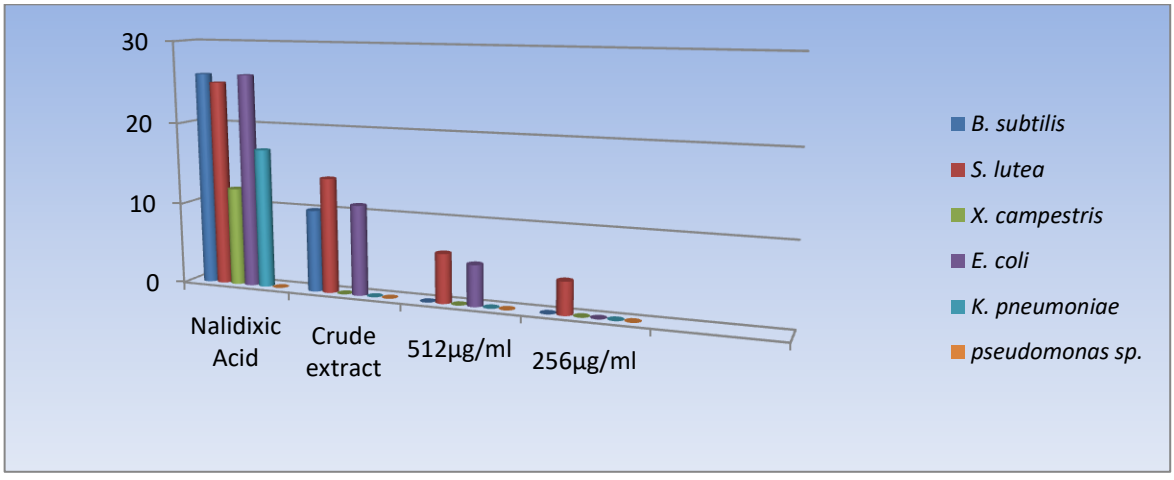

Fig 2: Antimicrobial activity of commercial disc (Nalidixic acid) and methanolic isolate of *C. obtusifolia* leaf.

Estimation of Antibacterial Activity of Ethanolic Extract of *C. obtusifolia* Leaf - From the **Table 3**, it has been shown that the leaf powder of the *C. obtusifolia* showed antimicrobial activity against some of the bacteria as well as *E. coli* (005-1), *K. pneumonia* (006-1) and *Pseudomonas sp*. (010-1). The crude extract of leaf powder produced 11mm area of inhibition against *K. pneumonia* (006-1). It also produced a area of inhibition of 7mm against of the organism of *Pseudomonas sp*. (010-1).

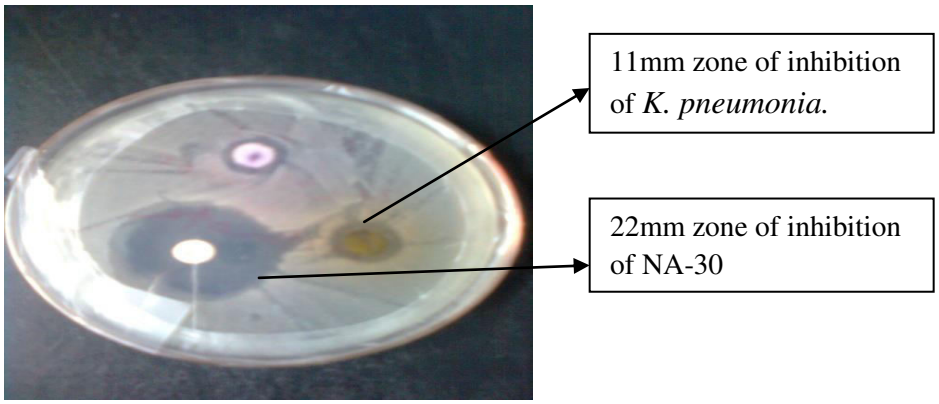

Fig 3: Zone of inhibition with methanol extract of *C. obtusifolia* leaf against *Sarcina lutea* (002-1).

Table 3: Zone of inhibition produced by ethanolic extracts of *C. obtusifolia* leaf against different bacterial strain.

Name of bacterial strain	**Diameter of zone of inhibition (mm)**									
	Commercial antibiotic disc (NA-30) (30 µg/µl)	**Crude Extract**	**Extract concentration (µg/ml)**							
			512	256	128	64	32	16	8	4	**O**
***B. subtilis* (001-1)**	12	-	-	-	-	-	-	-	-	-	**-**
***S. lutea* (002-1)**	31	-	-	-	-	-	-	-	-	-	**-**
***X. campestris* (004-1)**	16	-	-	-	-	-	-	-	-	-	**-**
***E. coli* (005-1)**	22	8	-	-	-	-	-	-	-	-	**-**
***K. pneumonia* (006-1)**	22	11	3	-	-	-	-	-	-	-	**-**
***Pseudomonas sp* (010-1)**	**18**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

N.B. (-) Not Inhibition, O- Negative control.

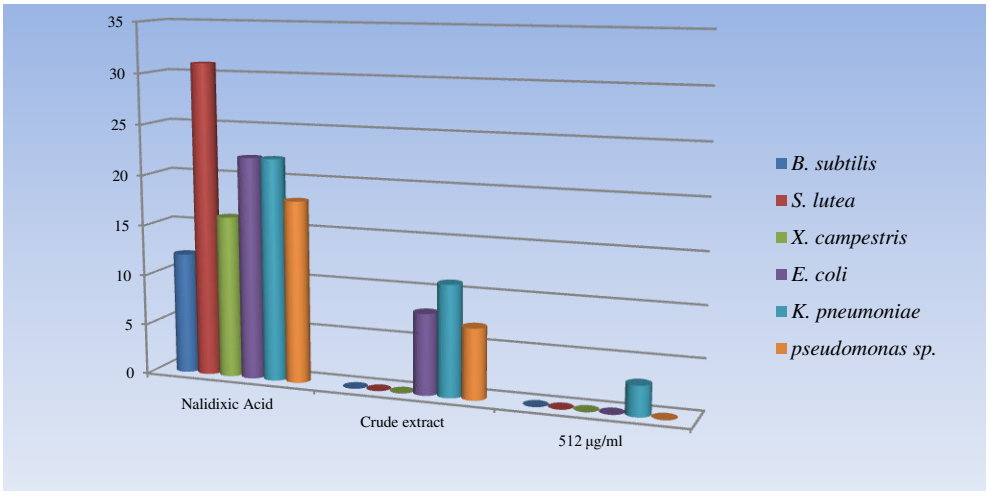

Fig 4: Antimicrobial activity of commercial disc (Nalidixic acid) and ethanolic extract of *C. obtusifolia* leaf.

MIC was noted in almost all of the bacterial strain when different extracts were used. For the ethanolic extract, the MIC of the *C. obtusifolia* leaf against *K. pneumonia* (006-1) was 512 µg/ml produced 3 mm area of inhibition (**Table 4**). For the methanolic isolate, the MIC of the *C. obtusifolia* leaf against two bacterial strains, viz., *S. lutea* (002-1), *E. coli* (005-1) was 256 µg/ml and 512 µg/ml that produced 4 mm, and 5 mm area of inhibition respectively (**Fig 5 & 6**).

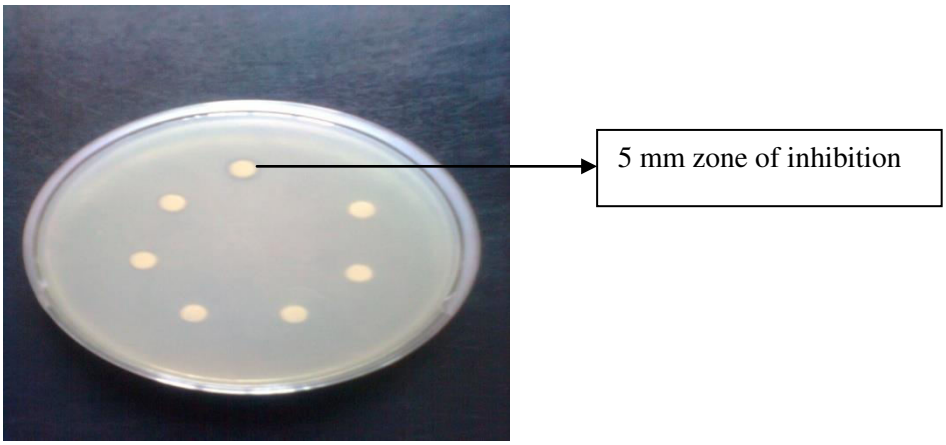

Fig 5: Observation of MIC of methanolic extract of *C. obtusifolia* leaf against *E. coli* (005-1).

Table 4: Comparison study of MIC of ethanol and methanol extract of *C. obtusifolia* leaf.

Name of bacterial Strain	Minimum Inhibitory Concentration (MIC)			
	Ethanolic extracts (µg/ml)	Zone of inhibition (mm)	Methanolic extract (µg/ml)	Zone of inhibition (mm)
***S. lutea* (002-1)**	512	0	256	**4**
***E. coli* (005-1)**	512	0	512	**5**
***K. pneumonia* (006-1)**	**512**	**3**	**512**	**0**

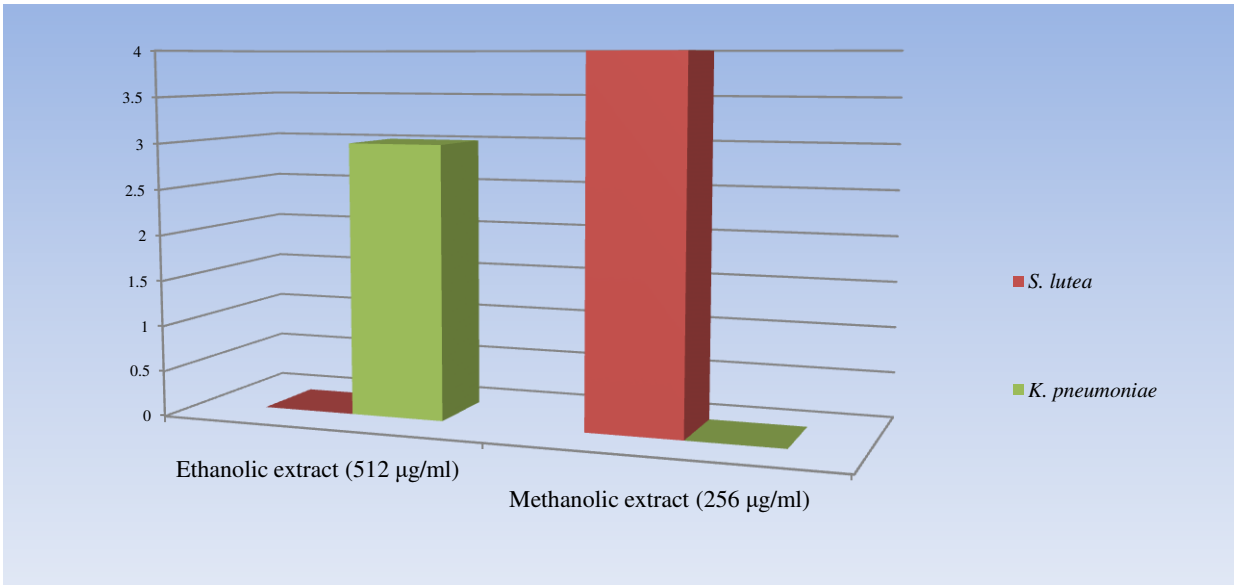

Fig 6: Comparative MIC study of ethanolic and methanolic extract of *C. obtusifolia* leaf.

DISCUSSION:

The present study was conducted to evaluate the effects of the organic extract of important medicinal plant *C. obtusifolia* leaf on selected pathogenic microorganisms. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made a significant contribution towards human health (Somchit *et al*., 2005). Phytomedicine can be applied for the treated of diseases as is done in case of Unani and Ayurvedic technique of medicines or it can be the base for the improvement of a drug. The presence of antimicrobial substances in higher herbs is well confirmed (Srinivasan *et al.*, 2001). Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made a significant contribution towards human health (Somchit *et al*., 2005). Sequential isolation of alkaloids from plant resource is largely host on the type of solvent used in the isolation process. The present healers apply initially water as the solvent (Rastogi *et al.,* 2002).

The increasing social and economic implication caused by pathogenic bacteria means there is constantly striving to develop new antibacterial agents. The command for more natural antimicrobial drug has driven researchers to analyze the inhibitory drugs such as isolates from plants (Sohn *et al.,* 2004). Various publications have documented the antimicrobial activity of plants extracts (Ahmad and Aqil, 2007; Hawang *et al*., 2004; and Mathabe *et al*., 2006). Thus plants extracts are promising natural antibacterial agents with pivotal roles in pharmaceutical industries for regulating the pathogenic microbes. As a medicinal plant, *C. obtusifolia* are beneficial in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, lung diseases, and heart disorders. In this present study, we tested the antimicrobial activity of *C. obtusifolia* leaf extract (with two different solvents, i.e., methanol and ethanol) against six pathogenic strain as well as *B. subtilis* (001-1), *S. lutea* (002-1), *X. campestris* (004-1), *E. coli* (005-1), *K. pneumoniae* (006-1) and *Pseudomonas sp* (010-1).

From this study it has been observed that the methanolic isolate of *C. obtusifolia* showed moderate antimicrobial activity against different pathogenic bacteria strain. The crude extract showed the highest area of inhibition (14 mm) against *S. lutea* (002-1) (**Fig 2**). It also produced 10 mm and 11 mm area of inhibition against *B. subtilis* (001-1) and *E. coli* (005-1) respectively (**Table 2**). From this study, it has also been observed that the ethanolic extract of this herb also showed potential antimicrobial activity against different tested pathogenic strain. The crude ethanolic extract of this herb showed moderate area of inhibition (11mm) against *K. pneumonia* (006-1) (**Fig 4**). It also produced 8 mm and 7 mm area of inhibition against *E. coli* (005-1) and *Pseudomonas sp.* (010-1) respectively (**Table 3**). It produced no area of inhibition against *B. subtilis* (001-1), *S. lutea* (002-1), *X. campestris* (004-1) and *Pseudomonas sp* (010-1) (**Table 3**).

For methanolic isolate, the MIC of the *C. obtusifolia* leaf was 256 µg/ml that produced a 4 mm area of inhibition against *S. lutea* (002-1) (**Table 2**). It also produced 6mm and 5 mm area of inhibition against *S. lutea* (002-1) and *E. coli* (005-1) respectively at 512 µg/ml concentration. For ethanolic extract, the MIC of the *C. obtusifolia* leaf against only *K. pneumonia* (006-1) was 512 µg/ml produced 3mm area of inhibition (**Table 4**). Different concentration *i.e.,* 256 µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml, 2 µg/ml of ethanolic and methanolic isolates of *C. obtusifolia* leaf produced no area of inhibition against pathogenic bacteria (**Table 2**, and **Table 3**). The MIC (256µg/ml) of methanolic isolate was found to be more effective between the two extracts (ethanol and methanol), against *S. lutea* (002-1) that produced a 4 mm area of inhibition. On the other hand, the MIC of methanolic isolate of this herb was 512 µg/ml that produced 3 mm area of inhibition against *K. pneumonia* (006-1). This herb is distributed mainly in China, Korea, India and the western tropical regions (Vadivel *et al*., 2012). In this study, Commercial antibiotic disc (Nalidixic Acid 30 µg/µl) was used as a positive control that showed antimicrobial activity against all of the tested pathogenic bacteria except *Pseudomonas sp.* (010-1). Negative control (disc containing only ethanol or

methanol solvent) exhibited no zone against the six bacterial strains. So; it has been concluded that methanolic isolate of *C. obtusifolia* showed better result in comparison to methanolic isolate against some selected human pathogenic bacteria.

CONCLUSION:

Medicinal plants are a rich source of antimicrobial agents which could be exploited in human disease prevention. From the observed results of the project work, it can be calculated that the *C. obtusifolia* leaves extracts, inhibit the growth of selective human pathogenic bacteria strain and possesses the potent antimicrobial activity against selective human pathogenic bacteria strain. Between the two solvents, the methanolic isolates of *C. obtusifolia* leaves were found to be more effective against selective human pathogenic bacteria than that of the extracts with ethanol. Ayssiwede *et al.* (2012) reported that Cassia leaves on a dry matter basis contain 27.4% crude protein, 16.8% crude fiber, ash 15.2%, 3.8% ether extract, 36.8% nitrogen-free extract and metabolizable energy of 2050.47 kcal/kg. In addition, it has been noted that *C. obtusifolia* and its elements have estrogenic activities and inhibit histamine secrete from mast cells (Wang *et al*., 2005b). More researches must be needed to identify the exact species that showed resistance against this plant extracts. The findings of our present study further revealed the antimicrobial activity of *C. obtusifolia* L., and the biological examination of these compounds also can support us to find a new application of traditional Bangladeshi herb.

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CONFLICTS OF INTEREST:

The authors declared no potential conflicts of the interest with the present study.

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