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ORIGINAL ARTICLE **OPEN ACCESS**

Antibacterial Activity of *Cyamopsis tetragonoloba* (Guar) against *E.coli* (Gram -ve) and *Bacillus subtilis* (Gram +ve)

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ABSTRACT
Medicinal plants have been used as medication for human diseases because they contain chemical components of curative value. Medicinal plants are good source to obtain a variety of therapeutic medicines. Dry powder of Guar seed was prepared by grinding method. Plant extract prepared through Soxhlet distillation method in Methanol and Ethanol organic solvents separately. The plant extract were prepared for antibacterial activity which is used against E.Coli and Bacillus subtilis by Disc diffusion method. Gentamycin disc and Streptomycin disc were used as control media. The result shows that Guar seeds are having good antibacterial activity and showing minimum inhibitory concentration zone of 23 mm against E. coli and 10 mm against Bacillus subtilis in Methanol solvent whereas 19 mm against E.coli and 7 mm against Bacillus subtilis in Ethanol solvent.
Keywords: *Cymopsis tetragonoloba, E. coli, Bacillus subtilis*

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INTRODUCTION

Cyamopsis tetragonoloba commonly known as guar bean or cluster bean. It is an annual legume belongs to family *Fabaceae,* and it is a good source of guar gum. Guar is domesticated in India and consumed like green beans vegetable. Young, fresh guar beans have narrow as well as long body together with small pods. They are extremely popular vegetable in people of India because of its medicinal values. It is broadly grown in many parts of country (dry, warm and arid regions). Fresh and young guar beans are collected for vegetable use. Now a days, it is grown more for mucilagenous gum production because it works as an appetizer and assimilating agent. It is also works in reducing purgative, dyspepsia and anorexia. and has tendency to act as anti-ulcer, anti cancerous, to reduce high glucose level, to lower high cholesterol. It also have antibacterial activity and antivermifuge activity and is used as curative agent of diabetes, asthma, and obesity [1, 8] **.**

Cyamopsis tetragonoloba L. contains high amount of proteins, carbohydrates and it lacks toxins. It also contains Macro and Micro nutrient like iron, sodium, potassium, calcium, magnesium, nickel, copper, cobalt and some vitamins [9, 10]. The endosperm of Guar beans contains galactomannan gum (a component of carbohydrate) when galactomann is mixed with water it forms gel. *Cyamopsis tetragonoloba* consist of natural gum, which is an edible thickening agent, which is extensively used in food and industrial purpose. It is used as a lenitive, purgative or aperients substance which is also used for treatment of loose motion, obesity and diabetes, and for lowering cholesterol level. Seeds of Guar are used in cure of asthma, night blindness and inflammation whereas the pods are used as an anti oxidant [5,6], and in treatment of arthritis [4]. This medicinal plant is used in treatment of dyspepsia or indigestion and further can be used as curative agent in constipation and anorexia, and aggravation of kapha and pitta. *C. tetragonoloba* Plant commonly known as Aperitif and Flatugenic [2].

In recent inventions shows that, multiple drug resistance (MDR) in human has been developed towards pathogenic bacteria because there are substantial use of antimicrobial medicine like antibiotics. In most of the cases antibiotics are commonly used in the treatment of infectious diseases which after continuous use developed MDR. In this situation scientists searched new antimicrobial molecule from various

medicinal plants which proves good curative agent for infectious disease. The problem of microbial resistance (MDR) is still growing continuously and the future prospects for the use of antimicrobial medicines cannot be diverted. So, there is a need to take proper actions to reduce MDR related problem. The use of plant extracts with some additional molecules and phytochemical substances both with known good antimicrobial properties can have great importance in therapeutic treatments. In this study, to determined the antioxidant and antimicrobial properties of Seed of *C. tetragonoloba* against *E.Coli* (Gram -ve) and *Bacillus subtilis* (Gram +ve).

MATERIAL AND METHODS

Collection of plant material:

The Seeds of *Cyamopsis tetragonoloba Beans* were purchased from the Market. The beans were then absolutely washed with tap water and then with distilled water, After that beans were dried under shade by covering with transparent cloth. The beans then crushed with the help of Mortal and Pastel and seeds were separated out. The dried seeds parts were integrated into fine powder and stored in air tight container which was stored for further used for solvent extraction.

Micro - organism used - *E.coli* (MTCC 294) and *Bacillus subtilis* (*MTCC* 441)

Solvent used - Ethanol and Methanol

Drugs and Chemical used-: Streptomycin and Gentamycin.

Preparation of plant Extract

The dry powder of bean *C. tetragonoloba* was prepare by Soxhlet Extraction method following**.**

Soxhlet Extraction is a laboratory apparatus. A Soxhlet extraction is used when the intended compound has a limited solubility in a solvent. About 40 gm of *C. tetragonoloba* bean powder material were being uniformly packed in to a thimble and move in Soxhlet extraction. It was depletable extracted with 200 ml Methanol and Ethanol solvents separately for the duration about 48 hour or 24 cycles until the solvent (Methanol and Ethanol) in the siphon tube of an extract become colour less. After completion of this process Seed extract was filtered with the help of Whatman filter paper. The bean extract were used against *E.coli* (Gram -ve) and *Bacillus subtilis* (Gram +ve) Bacteria for MIC and MBC. The residue left was dried over anhydrous $NaSO_4$ to remove left residue of alcohol. Then Seed extract kept in refrigerator at 4°C for detection of antibacterial activity and to examine their physical and chemical property.

Bacterial preparation and maintenance: Bacterial cultures maintained using nutrient agar and nutrient broth and bacterial culture maintained in the BOD incubator at temperature of 35-37ºC for 24-48 hours.

Disc Diffusion method [7]: The susceptibility testing of the plant extracts was calculated by using disc diffusion method and Minimum Inhibitory Concentration of the extract which were resolved by using a serial dilution method. *E.coli* and *B.subtilis* were grown in nutrient agar media for 18 hours before use, then inoculum suspensions systemized by 18 h culture at 37°C in 10 ml of Mueller Hinton Broth. The cultures were calibrated to around 119 CFU/ml with sterile saline solution, then 15ml of the suspensions spread over each plates containing Mueller-Hinton agar using a sterilized cotton swab and distribute them uniformly for microbial growth on petri plates. Again all were tested against the effect of the plant seed extracts at different concentration of 20ml mg/ml, 25mg/ml, 30mg/ml. These Petri plates were incubate for 24 hour at 37°C after that zone of inhibition (in millimeter) were recorded . The Minimum zone of inhibition of plant seed extract were compared with that of the standard antibiotic i,e Streptomycin and Gentamycin at a concentration of 1mg/ml [3]**.**

Serial Dilution Method: In vitro antimicrobial testing of the purified extract from *Cyamopsis tetragonoloba* seeds tested and established against the *E.coli* and *Bacillus subtilis* by using serial dilution method for minimum inhibitory concentration (MIC). This method is used in a number of different samples to determine the number of micro-organism that are present in a given population. In this method we take 5 test tubes, labelled each test tube as 10^{-1}, 10^{-2}, 10^{-3} 10^{-4}, 10^{-5}. In first test tube take 9 ml distilled water and 1ml *Cyamopsis tetragonoloba* seed extract sample. Continuously stir the extract suspension mixture thoroughly with the help of vortex. In rest of the test tube add 9 ml distilled water in each test tube. With the help of clean pipette, withdraw 1ml extract suspension from master test tube ($10^{-1)}$ and add into 2nd test tube. Continue this until the last test tube get the sample suspension.

Phytochemical Screening of Plant Extract

The phytochemical investigations for different biochemical compound like glycosides, alkaloids, flavonoids, glycosides, phenols, steroids, resins, saponins, tannins, terpenoids, triterpenoids and ascorbic acid were made by following standard procedures.

RESULT

Preliminary phytochemical analysis were recorded in Table 1.The response (MIC) is recorded in terms of zone of inhibition and inhibitory concentration. It is shown in the table-3 that Minimum zone of inhibition is around 23mm in *E.coli* and 19 mm in Bacillus at different dilution concentration of plant extract in methanol extract while 10mm in *E.coli* and 7 mm in *Bacillus subtilis* in ethanol extract were recorded.

Table 1: Phytochemical screening of C*yamopsis tetragonoloba* (seed)

S.No.	Solvent	Plant Part	Methanol	Ethanol
1	Alkaloids	S	+	-
2	Carbohydrates	S	+	-
3	Cardiac Glycosides	S	+	+
4	Proteins	S	+	-
5	Phytosterols	S	+	-
6	Flavonoids	S	+	+
7	Tannins	S	+	-
8	Terpenoids	S	+	+
9	Saponins	S	+	-
10	Phenols/ Polyphenols	S	+	+

Where, "+": presence, "-": Absence, "S": Seed

Table 2: Antimicrobial activity of seed extracts of *Cyamopsis tetragonoloba* against pathogenic microbes

S.No.	Solvent	Plant Part	Inhibition zone in (mm) against pathogenic microbes after 24 hrs incubation (*E.coli*)		
			0.75mg	0.5mg	0.25mg
1	Methanol	Seed	**23**±0.8	**22**±0.8	**18**±0.8
2	Ethanol	Seed	**10**±0.4	**8**±0.4	**6**±0.4

Table 3: Antimicrobial activity of Seed extracts of *Cyamopsis tetragonoloba* against pathogenic microbes

S.No.	Solvent	Plant Part	Inhibition zone in (mm) against pathogenic microbes after 24 hrs incubation (*Bacillus subtilis*)		
			0.75mg	0.5mg	0.25mg
1	Methanol	Seed	**19** ± 0.6	**17**± 0.6	**14** ± 0.6
2	Ethanol	Seed	**7** ± 0.2	**8** ± 0.4	**5** ± 0.2

Table 4. Antimicrobial potential of extracts against standard antibiotics.

Antibiotic	Dose (mcg)	Zone of inhibition (mm) against pathogenic agent (*E.coli*)
Streptomycin	10	36
Gentamycin	10	22

Table No.-5: ANOVA test showing standard deviation between the group and within the group

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	41.292	2	20.646	.375	.688
Within Groups	7756.458	141	55.010		
Total	7797.750	143			

DISCUSSION AND CONCLUSION

In the present investigation, the antimicrobial activity of Ethanolic and Methanolic extract of *Cymopsis tetragonoloba* were evaluated in which the antimicrobial activity of Methanol seed extract of guar showed minimum inhibitory antibacterial activity against *E. coli* and minimum antibacterial activity against *Bacillus subtilis* bacteria. The Methanolic and Ethanolic seed extract of *Cyamopsis tetragonoloba* gave the best results in the form of zone of inhibition. Methanol extract of *Cyamopsis tetragonoloba* gave it's as minimum size of zone of 23 mm in case of *E. coli* (0.50 mg/ml). In comparison to these results, in this study the *Bacillus subtilis* showed minimum zone of inhibition (19 mm) in Ethanol seed extract of

Cyamopsis tetragonoloba. Comparatively, in this study *E. coli* exhibited minimum zone of inhibition (23mm) in methanol and minimum zone of inhibition is (10nm) in ethanol While *Bacillus subtilis* exhibit minimum zone of inhibition (19nm)in methanol and minimum zone of inhibition is (7nm) in ethanol . In conclusion, significant inhibitory activity of methanol extract of guar gum was noted against pathogenic microorganisms *E.coli* and *Bacillus subtilis.* The Methanolic plant extract could be studied further as future alternatives to control diseases associated with pathogenic bacteria. Phytochemical analysis test could be carried out to isolate the biologically active compounds of this plant species, which act as antioxidant, antimicrobial, anti diabetic, anti-cancerous and anti obesity agents. These phytochemical compounds could be used to produce medicines which could be proved effective against certain type of disease.

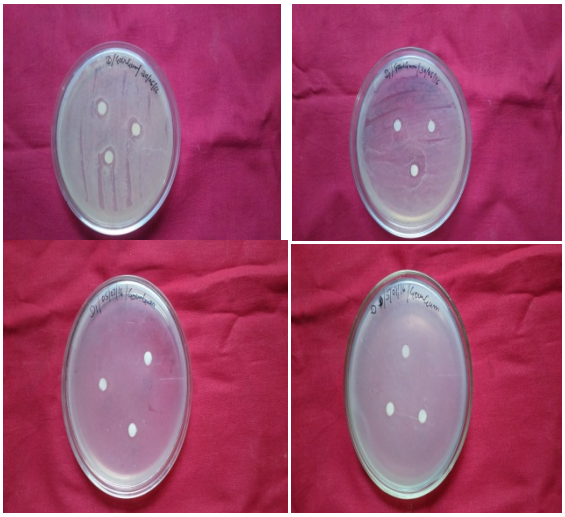

Fig.2- Antimicrobial activity of Methanolic Extract against *Bacillus subtilis*

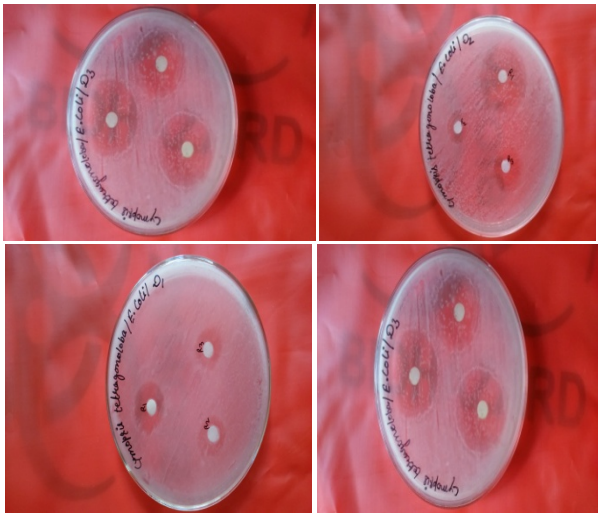

Fig.3-Antimicrobial activity of Methanolic Extract against *E.coli*

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