International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 16-08-2021, Accepted: 01-09-2021, Published: 17-09-2021
Volume 6, Issue 5, 2021, Page No. 616-621

Effect on anti-diabetic and antibacterial activities of polyphenol rich extract from the seeds of *Caesalpinia crista*

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Abstract
The aim of this study was to evaluate the antidiabetic and antibacterial, polyphenol rich extract from the seeds of *Caesalpinia crista*. Phytochemicals were studied through qualitative tests and phytochemical visualized by thin layer chromatography (TLC). Polyphenol rich extract from the seeds of *C. crista* were assessed for antibacterial potential via the agar well diffusion method against *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis* and *Pseudomonas aeruginosa*. The minimal inhibitory concentrations (MIC) were determined by the microdilution method. *In vitro* antidiabetic activity of the polyphenol rich extract from the seeds of *C. crista* was considered using inhibition of amylase and glucosidase assays. Polyphenol rich extract from the seeds of *C. crista* had the highest phenolic and flavonoid contents (66.78±1,89 µg GAE/g, 54,56±2.84µg QE/g. Polyphenol rich extract from the seeds of *C. crista* considerably inhibited the growth of the bacteria *S. aureus, E. coli* and *K. pneumonia*. The lowest EC_{50} value (76.32 and 71.32 µg/mL) observed in the inhibition of amylase and glucosidase. The study therefore showed that the polyphenol rich extract from the seeds of *C. crista* possess significant antibacterial and inhibitory effect on glucose diffusion in vitro thus affirming the traditional claim of the use of plant in treating bacterial Infection and diabetes.

Keywords: antibacterial, antidiabetic, polyphenol rich extract; *C. crista*

Introduction

Plants and plan-based products have been used traditionally by the inhabitants of India from the time immemorial. Several references of healing properties of plants are stated in Rigveda (400-1500 B.C.) Atharvaveda (1500 B.c.). Some of the *in vivo* free radicals play a positive role in phagocytosis, energy production and regulation of cell growth etc. However, free radicals may also be destructive. Free radicals produced in the body react with various biological molecules namely lipids, proteins and deoxyribonucleic acids resulting in the imbalance between oxidants and antioxidants. Even though our body is safeguarded by natural antioxidant defense, there is always a demand for antioxidants from natural sources (Rimbach *et al.*, 2005) [9]. Phenolic compounds from medicinal plants possess strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free-radicals (Kahkonen *et al.*, 1999). They are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers (Proestos *et al.*, 2006) [8]. Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases (Lai and Chou, 2001) [6].

Diabetes mellitus (DM) is a serious, chronic, and complex metabolic disorder of multiple aetiologies with profound consequences, both acute and chronic (Soumya and Srilatha, 2011) [10]. Also known only as diabetes, DM and its complications affect people both in the developing and developed countries, leading to a major socioeconomic challenge. It is estimated that 25% of the world population is affected by this disease. There are several classes of oral hypoglycemic drugs that exert antidiabetic effects through different mechanisms, namely sulfonylureas, biguanides, α-glucosidase inhibitors, thiazolidinediones, and non-sulfonylureas secretagogues. Oral sulfonylureas, such as glimepiride and glyburide, act to reduce blood sugar, mainly by elevating insulin release from islets of Langerhans. This is achieved through binding with the sulfonylurea receptor on β cells resulting in adenosine triphosphate-dependent potassium channels closure. As a result, the cell membrane depolarizes and the following calcium influx accompanied by secretion of stored insulin from secretory granules within the cells takes place. This mechanism works only in the presence of insulin (DeFronzo, 1999) [4].

Antimicrobial agent possesses diverse chemicals structure and varies in their assortment of action. Some act on both Gram positive and Gram negative bacteria and they are said to have a broad spectrum of action, while some are only strong on either Gram positive and negative bacteria are said to be constricted, examples of narrow spectrum antibiotics (Meenaa *et al.*, 2009) [7]. Antibacterial are definitely effective in killing bacteria, however, there is considerable controversy surrounding their health benefits such as toxicity to some organs or harmful to some normal body floras. The non-residue producing agents have been used for many years and continue to be effective agents for controlling disease organisms in a wide variety of healthcare and domestic settings. When used under strict guidelines of application, the residue-producing agents have proven effective at controlling bacterial and fungal infection in clinical settings such as hospitals (Ahmed and Urooj, 2010) [1]. *Caesalpinia crista (Caesalpiniaceae)* is a large scandant

prickly shrub found throughout the interior parts of India. It is common in southern parts of India and is often grown as a hedge plant. This plant has recorded medicinal use and is demonstrated to have antibacterial activity, antidiabetic activity anthelmintic, anti-inflmmatory, antipyretic, analgesic, anti-amyloidogenic, antioxidant, hypoglycemic activity and hepatoprotective activities.

Materials and Methods

Plant Material

The seeds of *Caesalpinia crista* were obtained from Herbal garden of Government Siddha Medical College, Arumbakkam, Chennai, Tamil Nadu, India. A plant taxonomist authenticated the plant and samples were kept in the Medicinal Botany herbarium with voucher specimen numbers MB/GSMC-255/2021. The flowers were sufficiently air-dried in 5 days at the ambient room temperature, while the flower was cut into smaller pieces and air-dried in 7 days.

Phytochemical Screening

The aqueous extract of *Caesalpinia crista* seeds were subjected to phytochemical screening to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols using standard procedures (Aida *et al*., 2001; Hess *et al*., 1995).

Extraction of Polyphenols

Polyphenols were extracted from crushed seeds of *C. crista* (100 g), according to the method of Zhang *et al*. (2000). The fraction was completed twice at 20 °C in a shaking incubator. Methanol/acetone/water (3.5:3.5:3, v/v/v) containing 1 % formic acid were used extracting solvents were 100 mL at 30 min. The extract was then filtration through Whatman No.1 filter paper. The filtrates solutions were evaporated under vacuum at 40 °C to remove methanol and acetone. Lipophilic colours materials were removed from the aqueous phase by two consecutive extractions in a separator funnel with a twofold volume of petroleum ether. The aqueous phase was finally collected and further extracted three times by ethyl acetate (ethyl acetate: aqueous phase = 1:1, *v/v*) in the separator funnel. The ethyl acetate phases were collected, evaporated and dried under vacuum at 35 °C to obtain polyphenol sample.

Thin layer chromatography

The polyphenols were extracted from the seeds of *C. crista* were loaded on to pre coated TLC (60 F_2 54) and it was developed using solvent system in the ratio of Petroleum ether, Chloroform and methanol (1:0.5:0.1, V/V/V) was used for the development of the exudates on silica gel plates silica gel 60 F_{254} (10x20 cm, 0.2mmlayer). Visible and the non-visible spot given and it is fluorescent with UV light at 360nm and 240nm.

In vitro evaluation of yeast cell uptake of glucose

Commercial baker's yeast was wash by repeated centrifugation (3,000×g,5min) in distilled water until the supernatant fluid were clear and a 10% (v/v) suspension was prepared in distilled various concentration of extracts (25-100 µg/ml) were added to 1mL of glucose solution (25 mM) and further incubated for 10min at 37 °C. reaction was started by adding 100µl of yeast suspension, vortex and further at 37 °C at 60min, the tubes were centrifuged (2,500×g, 5min) and glucose was estimated in the supernatant (Cirillo, 1962) [3], metformin was taken as standard anti-diabetic drug used. The percentage of increase in glucose uptake by yeast cells was calculated using the following formula:

Inhibition of A-Amylase Enzyme

α-amylase (0.5 mg/ml) was mixed with the polyphenols were extracted from crushed seeds of *C. crista* at various concentrations (25-100 µg/ml) to which 1% of starch solution and 100 µl of 0.2 mm of phosphate buffer (pH -6.9) were added. The reaction was allowed to be carried out at 37°C for 5 min and terminated by addition of 2 ml of 3, 5-dinitrosalicylic acid reagent. The reaction mixture was heated for 15 min at 100°C and diluted with 10 ml of distilled water in an ice bath. α-amylase activity was determined by measuring color intensity at 540 nm in spectrophotometer.

Inhibition of A-Glucosidases Enzyme

The inhibitory activity was determined by incubating 1 ml of starch solution (2% w/v maltose) with 0.2 M tris buffer (pH 8) and various concentration of polyphenols were extracted from crushed seeds of *C. crista* (25-100 µg/ml). The reaction mixture was incubated at 37°C for 10 min. The reaction was initiated by adding 1 ml of α-glucosidase enzyme (1 U/ml) to it and incubation at 35°C for 40 min. Then the reaction was terminated by the addition of 2 ml of 6 N HCl. The intensity of the color was measured at 540 nm in spectrophotometer. The results were expressed as % inhibition using the formula:

% inhibitory activity = (Ac-As)/Ac ×100

Where, Ac is the absorbance of the control and As is the absorbance of the sample.

Culture collection and maintenance

The bacterial strains of *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa.* These standard strains were obtained from Microbial Type Culture Collection and gene bank (MTCC); Institute of Microbial Technology, Chandigarh, India. The stock culture was maintained on Mueller Hinton agar medium at 4 ◦C.

Antibacterial activity

The antibacterial activities of the polyphenol rich fraction were assayed using the disc diffusion method (Drago *et al.*, 1999). Bacteria were grown overnight on Mueller Hinton agar plates, five colonies were suspended in 5 ml of sterile saline (0.9%) and the bacterial population in the suspension was adjusted to ~$3x10^8$ CFU/ml. A sterile cotton swab was dipped into the suspension and the swab rotated several times with firm pressure on the inside wall of the tube to remove the excess fluid. The swab was used to inoculate the dried surface of MH agar plate by streaking four times over the surface of the agar, rotating the plate approximately by 90º to ensure an even distribution of the inoculums. The medium was allowed to dry for about 3 min before adding a sterile disc of 6 mm diameter. Each disc was placed firmly on to the agar to provide uniform contact with the bacteria. Bioactive compound (50 µg) was weighed and dissolved in 1 ml of 7% ethyl acetate. The different concentrations of polyphenols were extracted from crushed seeds of *C. crista*

was introduced on to each disc and the control disc received only 7% ethyl acetate. The plates were incubated at 37°C for 24 h and the inhibition zone was measured and calculated. The experiments were carried out in duplicate three times. The results (mean value, *n*=3) were recorded by measuring the zones of growth inhibition surrounding the discs.

Minimum Inhibitory Concentrations (MICS)

The minimum inhibitory concentrations of the isolated compounds were determined by dilution method (Brantner and Grein, 1994). The strains were grown in Mueller Hinton broth to exponential phase with an *A*560 of 0.8, representing 3.2×10^8 CFU/ml. Different dilutions of the polyphenols were extracted from crushed seeds of *C. crista* were prepared to give solutions of 25, 50, 75, and 100 µg/ml. 0.5 ml of each concentration was added into separate test tubes containing 4ml of MH broth inoculated with 0.5 ml bacterial suspension at a final concentration of 10^6 CFU/ml. Each MIC was determined from five independent experiments performed in duplicate. The tubes containing 4.5 ml of bacterial inoculates and 0.5 ml of 7% ethyl acetate used as bacterial control, 4.5 ml of uninoculated MH broth and 0.5 ml PBS served as a blank.

The tubes were incubated at 37 ◦C for 18 h; inhibition of bacterial growth was determined by measuring the absorbance at 560 nm.

Statistical Analysis

Values were recorded as mean ± standard error of the mean. Statistical difference between the means was determined by one-way ANOVA followed by Duncan multiple range test.

Result and Discussion

Phytochemical Screening

The phytochemical screening of aqueous seed extract of *C. crista* studied presently showed the presence of alkaloids, flavonoids, polyphenol, terpenoids, and absence of glycosides and tannin (Table -1).

Table 1: Phytochemical screening of aqueous seed extract of *C. crista*

Sl. No.	Phytochemical Constituents	Observation	Aqueous seed extract of *C. crista*
1	Alkaloids	Orange /red precipitate	+
	▪ Dragendorff's Test-Mayers test	Yellow or white precipitate	+
2.	Flavonoids	Intense yellow colour	+
	▪ Alkalai Reagent-Lead acetate test	Precipitate formed	+
3.	Glycosides Keller	Reddish brown colour	-
	▪ Killiani test	ring formed	
4.	Tannin		
	▪ $FeCl_3$ test	Blue black coloration	-
5.	Saponins		
	▪ Frothing test	Foam	+
6.	Terpenoids	Dark reddish brown	-
	▪ *Salkowski test*	color in interface	
7.	Polyphenols		
	▪ Ferrozine test	Raddish blue	+
8.	Anthocyanin test Ammonia	Ammonia layer yellow in color	+

+ indicate positive result; - Indicate negative result

The Partial Characterization of Polyphenol Rich Extract From the Seeds of *C. Crista* by TLC

The polyphenol rich extract from the seeds of *C. crista* loaded on Pre-coated TLC plates (60 F_2 54 Merck) and developed with a solvent system of petroleum ether, chloroform and methanol in the ratio of 1:0.5:0.1 were efficient to extract the antidiabetic, antioxidant and anti-inflammatory compound it is used for further studies. The developed plate was viewed under UV 240nm and 360nm (Fig-1).

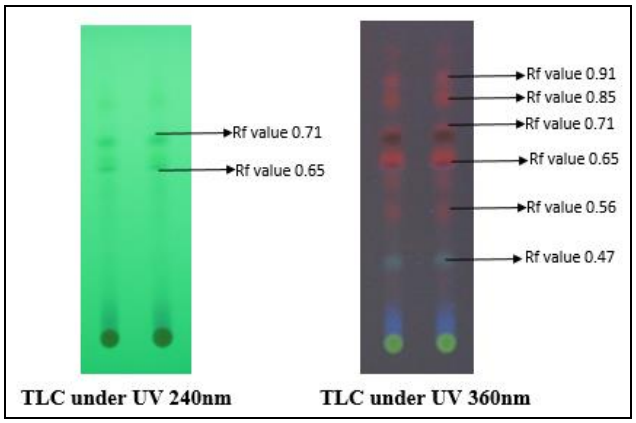


Fig 1: Partial characterization of polyphenol rich extract from the seeds of *C. Crista* by TLC

Glucose Uptake in Yeast Cells

Different concentrations of polyphenol rich extract from the seeds of *C. Crista* were subjected to *in vitro* glucose uptake assay employing yeast as model. The percentage of glucose uptake in yeast cells by the polyphenol rich fraction was compared with standard drug diclofenac sodium (Fig-2). Polyphenol rich extract from the seeds of *C. crista* exhibited highest percentage of glucose uptake 73.64%, which was

almost near to the standard 71.23% at 100 µg/ml concentration. Results also indicated that alkaloid rich fraction had nearly same effectiveness in increasing the glucose uptake by yeast cells as compared to standard drug acarbose. Polyphenol compound found chlorogenic acid is a natural in many varieties of plant species. It stimulates glucose transport in skeletal muscle via AMPK activation. Chlorogenic acid has shown effects on hepatic glucose release and glycemia (Bassoli *et al*., 2008) [2].

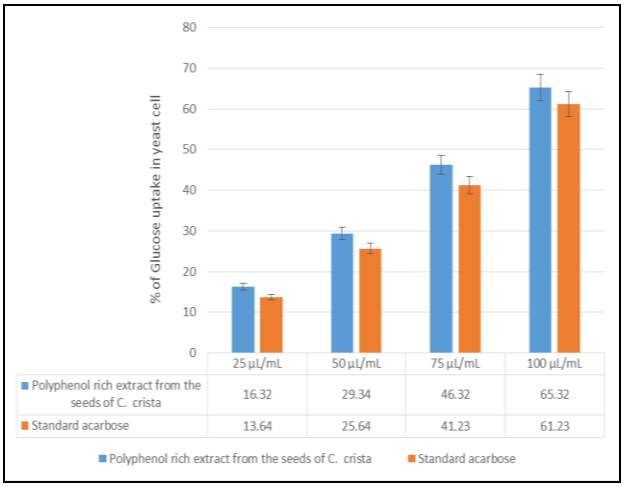


Fig 2: Glucose uptake in yeast cells by polyphenol rich extract from the seeds of C. crista

Alpha-Amylase Inhibition

Inhibitory effects of α-amylase confirmed that polyphenol rich extract from the seeds of *C. crista* at concentrations of 25-100 µg/ml (Fig. 3). The maximum inhibition was observed at highest concentration of 100 µg/ml exhibited of 79.36% as compared to standard acarbose which showed significantly lower inhibition of 74.65% at the same concentration. Alpha-amylase is type of the intestinal enzyme which play important role in carbohydrate digestion and glucose absorption (Worthington, 1993). Since alkaloid rich fraction, further studies have to conduct on the isolation, and characterization of the compounds in authority for the activity. Vasconcelos *et al*., (2011) [12]. Reported that aqueous extract of the stem bark of *Caesalpinia ferrea* (300 and 450 mg/kg, daily for four weeks) was administered orally to streptozotocin-induced diabetic rats.

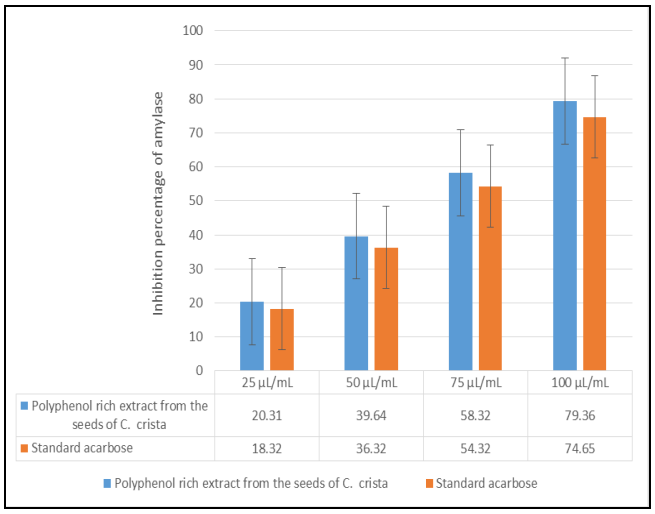


Fig 3: Inhibition percentage of alpha-amylase by polyphenol rich extract from the seeds of C. crista

Alpha-Glucosidase Inhibition

Another results of antidiabetic activity using α- glucosidase inhibitory assay of the polyphenol rich extract from the seeds of *C. crista* are shown in Fig-4. The polyphenol rich fraction revealed a significant inhibitory action of α-glucosidase enzyme. The percentage inhibition ranges from 21.23% to 83.35% for lowest concentration to highest concentration. Thus the inhibition of the activity of α-glucosidase by polyphenol rich extract from the seeds of *C. crista* desired interruption the degradation of carbohydrate. Similarly, Sun *et al*. (2015) [11] studied the antidiabetic and anticholinergic effects of chrysin on cyclophosphamide-induced multiple organ toxicity in rats by focusing on pharmacological evaluation of some metabolic enzyme activities: chrysin exhibited an ameliorative effect against CYP-induced brain, heart, liver, testis, and kidney toxicity.

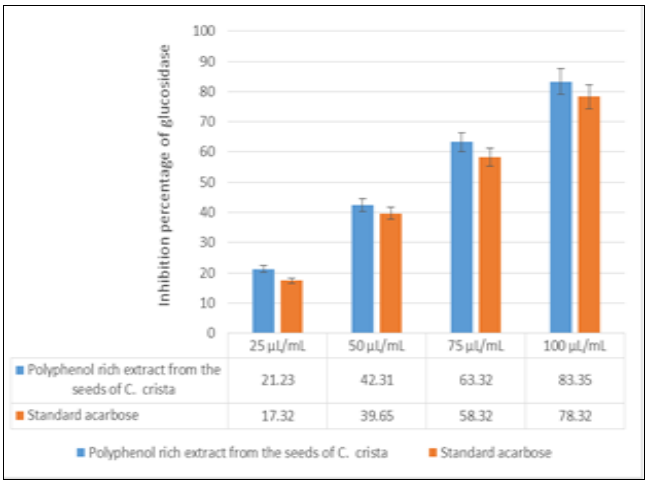


Fig 4: Alpha lucosidese inhibition by polyphenol rich extract from the seeds of C. cristo

Effect of polyphenol rich extract from the seeds of *c. crista* on the growth of pathogenic bacteria by disc diffusion method

Antibacterial activity of polyphenol rich extract from the seeds of *C. crista* tested against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* were screened and assayed as inhibition zones in the agar plates (Table-2). Upon testing all the bacteria tested were found to be sensitive to the polyphenol rich extract from the seeds of *C. crista*. Furthermore, the zone of inhibition study revealed that the polyphenol rich fraction possessed antibacterial activity in proportion to concentration gradient ranges 25-100 µl/ml against the tested microbes. Among the bacteria studied, *Staphylococcus aureus* (Gram positive) and *Escherichia coli* was diagnosed to be highly susceptible followed by *Pseudomonas aeruginosa* and *Enterococcus faecalis*. This may confirm the antibacterial property of polyphenol rich extract from the seeds of *C. crista*.

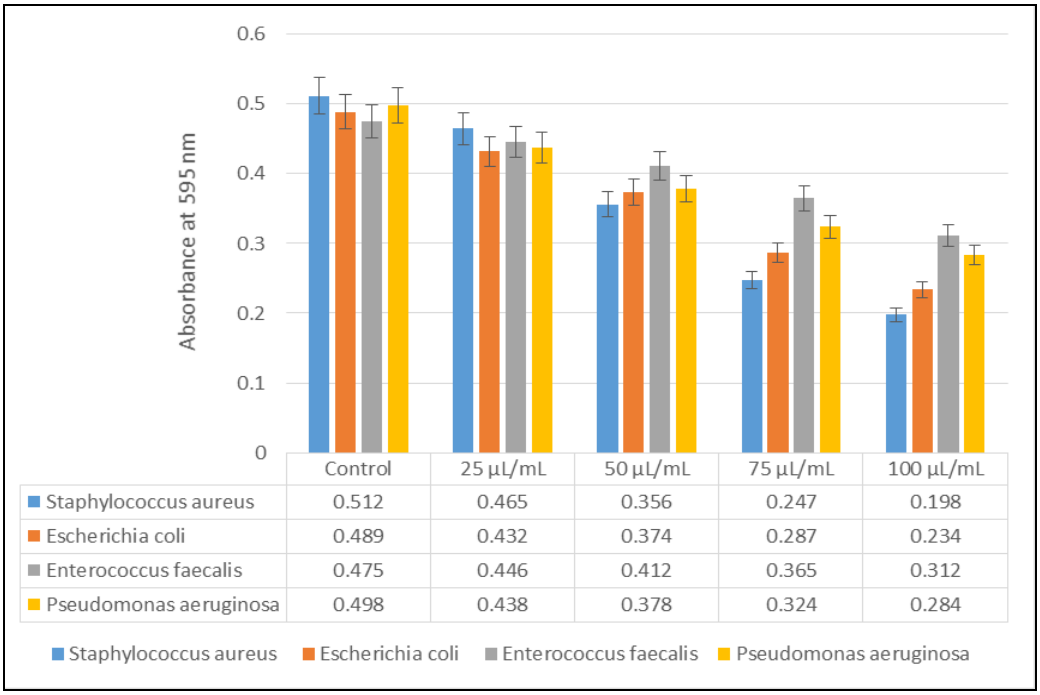
Table 2: The antibacterial activity of the polyphenol rich extract from the seeds of *C. crista* by disc diffusion method

Pathogenic organism	Different concentrations Crude extract (µl/ml)			
	25 µl/ml	50 µl/ml	75 µl/ml	100 µl/ ml
Staphylococcus aureus	8.5±0.1	12.3±0.9	15.6±1.1	17.6±1.3
Pseudomonas aeruginosa	6.2±1.3	9.3±0.6	12.3±1.6	14.3±2.1
Escherichia coli	8.3±0.5	10.3±0.6	13.4±0.7	15.1±0.3
Enterococcus faecalis	7.2±1.3	10.1±0.3	12.8±0.6	14.9±0.1

The inhibitory Zone size measured included the 6.0 mm size of the well by means of caliper. All the assays were duplicated, and the mean values were recorded.

Minimum Inhibitory Concentration

In the comprehensive arrangements, the MIC of polyphenol rich extract from the seeds of *C. crista* ranged between 25 to 100 µg/ml against gram positive bacteria and gram negative bacteria, (*Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa*) respectively. The Minimum inhibitory absorption value of polyphenol rich extract from the seeds of *C. crista* increases with increase in concentration. *S. aureus* and *E. coli* exhibited maximum inhibition when compared to the other pathogenic bacteria at 100 µl/ml concentration. *Enterococcus faecalis* and *P. aeruginosa* appearances reasonable range of inhibition activity. In comparison with gram positive bacteria and gram negative bacteria, the MIC of polyphenol rich extract from the seeds of *C. crista* displayed highest inhibition in gram negative bacteria and among the gram positive bactertia *S. aureus* showed maximum inhibition (Fig-5).



	Control	25 µL/mL	50 µL/mL	75 µL/mL	100 µL/mL
Staphylococcus aureus	0.512	0.465	0.356	0.247	0.198
Escherichia coli	0.489	0.432	0.374	0.287	0.234
Enterococcus faecalis	0.475	0.446	0.412	0.365	0.312
Pseudomonas aeruginosa	0.498	0.438	0.378	0.324	0.284

Fig 5: Antibacterial activity using MIC method by polyphenol rich extract from the seeds of C. crista

Conclusion

The present effort showing that polyphenol rich extract from the seeds of *C. crista* were analyzed for antidiabetic and antibacterial activity. The conclusions of the present study revealed that polyphenol rich extract from the seeds of *C. crista* have significantly bioactive poly phenolic compounds that showed higher potential as an antidiabetic effect antibacterial. Therefore, polyphenol rich extract from the seeds of *C. crista* can be recognized as a harmless and proficient for utilized as antibacterial, antidiabetic agent and an authoritative source for plant drugs synthesis and lessen the problem of multiple drug resistance particularly in case of pathogenic bacterial strains.

Acknowledgement

We thank Mr. SaiPrakash Leomuthu, CEO Sairam Institutions, Mr. SathishKumar CBO Sairam Institutions. Dr. S. Mathukumar M. D. (S), Principal Sri Sairam Siddha Medical College West Tambaram for Support and Encouragement to carry out the study.

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