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# Invitro antidiabetic and antibacterial properties of flavonoid rich extract from the stem bark of *Toona ciliata*

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**Abstract**
Diabetes, a chronic metabolic disorder, is characterized by persistent hyperglycemia. This study aimed to evaluate the hypoglycemic and antibacterial activities of Flavonoid rich extract from the stem bark of *Toona ciliata*. The hypoglycemic activity of the isolated strains was examined by evaluating the α-glucosidase and α-amylase inhibitory activities. The antibacterial activity was measured using the disc diffusion and MIC. Flavonoid rich extract from the stem bark of *Toona ciliata* exhibited potent α-amylase inhibitory (67.23%) and α-glucosidase inhibitory (74.32%) activities, which were comparable to those of acarbose (100 µg/mL). Therefore, present study suggest that the flavonoid rich extract from the stem bark of *Toona ciliata* with hypoglycemic, antibacterial properties can potentially prevent diabetes and infective person.

**Keywords:** *Toona ciliata;* antidiabetic; antibacterial; flavonoid rich fraction

## Introduction

Plants use as food and in traditional medicine are more likely to yield pharmacologically active compounds. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent therapeutic efficacy and antioxidant activities, no side effects and economic viability. Medicinal plants are serving as raw material for drugs which are effective and reasonable health care for people. However, all plants synthesize phytochemicals, which are beneficial for our health as they cannot be synthesized in the human body (Martinez *et al*., 2009). Plants are also rich dietary sources of biomolecules, vitamins and minerals which are crucial for maintaining the healthy body. It has been observed that numerous plants have pharmacological effects due to the presence of metabolites. Plant-metabolites are organic compounds which can be classified into primary metabolites and secondary metabolites. Primary metabolites are organic compounds include glucose, starch, polysaccharide, protein, lipids and nucleic acid which are beneficial for growth and development of the human body.

Diabetes mellitus is the most prevalent metabolic syndrome world-wide with an incidence varying between 1 to 8%. The disease arises when insufficient insulin is produced, or when the available insulin does not function properly. Thus diabetes is characterized by hyperglycaemia (elevation in blood sugar levels) resulting in various short-term metabolic changes in lipid and protein metabolism and long-term irreversible vascular changes. The long-term manifestation of diabetes can result in the development of some complications, broadly classified as microvascular or macrovascular disease. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethnobotanical information reports about 800 plants that may possess anti-diabetic potential (Alarcon-Aguilara *et al*., 1998) [1]. Several such herbs have shown anti-diabetic activity when assessed using presently available experimental techniques (Jafri *et al*., 2000) [8]. Wide arrays of plant derived active principles representing numerous chemical compounds have demonstrated activity consistent with their possible use in the treatment of diabetic II (Bailey and Day, 1989) [2].

*Toona ciliata* belongs to the family Meliaceae. The plant is found in Sub Himalayan tract of India, Assam and through-out hilly regions of central and southern India. The plant of *Toona ciliata* is a medium sized to large deciduous tree. The bark of plant is brown to grey and leaves are 15-45 cm long, usually paripinnate but sometimes with a terminal leaflet. Apex is acuminate, Base is strongly asymmetric, with entire margin (± toothed in saplings), mostly glabrous, Petals are 5-6 mm long and white in colour, seed of Toona ciliata is encapsulated in a ellipsoid capsule (10-20 mm long, 6-8 mm diameter), Seeds are winged at both ends. *Toona ciliata* stem bark flavonoids exhibit several biological effects such as antihepatotoxic, anti-inflammatory and anti-ulcer activity. They also inhibit enzymes such as aldose reductase, cycloxygenase, Ca ATPase, xanthine oxidase, phosphodiesterase and lipoxygenase.

Flavonoids are ubiquitous in photo synthesising cells and are commonly found in fruit, vegetables. Flavonoids, low molecular weight polyphenols of plant origin are a group of naturally occurring compounds. These are widely distributed in the human food supply through fruits and vegetables and are considered to bear potential anticarcinogenic effects. These are believed to be good scavengers of free radicals. Around 28 naturally occurring and synthetic flavonoids have been suggested as novel anti leukamic compounds. Besides, flavonoids have also been reported to exert multiple biological effects including anti-inflammatory anti allergic, antiviral and anticancer activity (Sharoni *et al*., 2000) [13]. In this study, the antidiabetic and antibacterial activities of flavonoid rich fraction from *Toona ciliata* stem bark inhibition of amylase, glocosidase and

against pathogenic bacteria, their possible modes of action, and potential applications were studied.

## Materials and methods

### Plant collection and preparation of extracts

*Toona ciliata* stem bark was 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-287-6/2021.

### Phytochemical analysis

The aqueous extract of *Toona ciliata* stem bark 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 (Harborne 1973).

### Glucose uptake in yeast cells

The commercial baker's yeast in distilled water was subjected to repeated centrifugation (3,000×g, 5 min) until clear supernatant fluids were obtained and a 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of the flavonoid rich extract from the stem bark *Toona ciliata* were added to 1mL of glucose solution (25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µL of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and amount of glucose was estimated in the supernatant (Dileep *et al*., 2018). Metronidazole was used as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

$$\text{Increase in glucose uptake (\%)} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$$

### Sample

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

### α-amylase inhibitory activity

α-amylase inhibitory activity of the strains was evaluated as described by Vankudre *et al.* (2015) [15]. Briefly, 250 µL of flavonoid rich extract from the stem bark *Toona ciliata* was added to 250 µL of α-amylase solution (0.5 mg/mL) and pre-incubated at 25 °C for 10 min. The reaction mixture was then incubated with 250 µL of starch solution (1% w/v in 0.02 M sodium phosphate buffer) at 25 °C for 10 min. Next, the reaction was terminated with the addition of 500 µL of DNS color reagent (96 mM DNS and 5.31 M sodium potassium tartrate in 2 M sodium hydroxide solution). The reaction mixture was then boiled for 5 min, allowed to cool, and diluted four-fold with water. The absorbance was measured at 540 nm. The inhibition was calculated as follows:

Inhibition(%)=[(A−B)A]×100,Inhibition(%)=[(A−B)A]×100,

Where *A* is the absorbance of the control and *B* is the absorbance of the sample.

### α-Glucosidase inhibitory activity

α-glucosidase inhibitory activity of the strains was measured according to the methods described by Chen *et al*. (2014) [4]. Briefly, 25 µL of flavonoid rich extract from the stem bark *Toona ciliata* was added to a reaction mixture containing 150 µL of 0.01 M PBS (pH 7.0) and 75 µL of 0.02 M PNPG solution, and pre-incubated at 37 °C for 10 min. The reaction was initiated with the addition of 50 µL α-glucosidase (0.17 units/mL) and the sample was incubated at 37 °C for 10 min. Next, the reaction was terminated with the addition of 1 mL of 0.1 M $Na_2CO_3$. The amount of p-nitrophenol released was determined by measuring the absorbance at 405 nm. The inhibition was calculated as follows:

Inhibition(%)=[1−(C−D)(A−B)]×100,Inhibition(%)=[1−(C−D)(A−B)]×100,

Where *A* is the absorbance with α-glucosidase but without sample, *B* is the absorbance without α-glucosidase and sample, *C* is the absorbance with α-glucosidase and sample, and D is the absorbance without α-glucosidase but with the sample.

### Antibacterial activity of flavonoid rich extract from the stem bark *Toona ciliata*

The antibacterial activities of the polyphenol rich fraction were assayed using the disc diffusion method (Drago *et al.*, 1999) [6]. 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 concentration of flavonoid rich extract from the stem bark *Toona ciliata* was introduced on to each disc and the control disc received only 7% ethanol. 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) [3]. 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 flavonoid rich extract from the stem bark *Toona ciliata* 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

Results are presented as the means ± standard deviation (SD) of experiments performed in triplicate. Graphical representation was generated using Prism software 9.0 (GraphPad Software, CA, USA). Statistical analysis was conducted using one-way analysis of variance (ANOVA) using SPSS (IBM Corp., USA).

## Result and discussion

### Phytochemical screening

The phytochemical screening of the aqueous extract from the *Toona ciliata* studied presently showed the presence of alkaloids, flavonoids, phenol, Terpenoids, glycosides and saponin, and absence of glycosides and tannin (Table-1).

**Table 1:** Phytochemical screenings of aqueous extract from the petals *Toona ciliata*

| Sl. No. | Phytochemical Constituents | Observation | Aqueous extract of *Toona ciliata* |
|---|---|---|---|
| 1 | Alkaloids<br>-Dragendorff's test<br>-Mayers test | Orange /red precipitate<br>Cream pie ppt | +<br>+ |
| 2. | Flavonoids<br>-Alkalai Reagent<br>-Lead aceate test | Intense yellow colour<br>Precipitate formed | +<br>+ |
| 3. | Glycosides- Keller-Killiani test | Pink colour (Ammonia layers) | + |
| 4. | Tannin-$FeCl_3$ test | Blue-black colour | + |
| 5. | Saponins- Frothing test | Foam | - |
| 6. | Terpenoids-Salkowski test | Reddish brown colour ring formed in interface | - |
| 7. | Polyphenols- Ferrozine test | Raddish blue | + |
| 8. | Anthocyanin- Ammonia test | Pink color in ammonia layer | + |

+ Positive result; - Negative result

### The partial characterization of flavonoid rich extract from the petals of *Toona ciliata* by TLC

The flavonoid rich extract from the stem bark *Toona ciliata* 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 not shown).

### Glucose uptake in yeast cells

The rate of glucose transport across cell membrane in yeast cells system is presented in Fig-2. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. The rate of uptake of glucose into yeast cells was linear in all the three glucose concentrations. The flavonoid rich extract from the stem bark *Toona ciliata* exhibited significantly higher activity than at all concentrations. However the highest uptake of glucose was seen in 20mM Glucose concentration. The result showed the lower uptake of glucose by the yeast cells which conformed the highest activity.

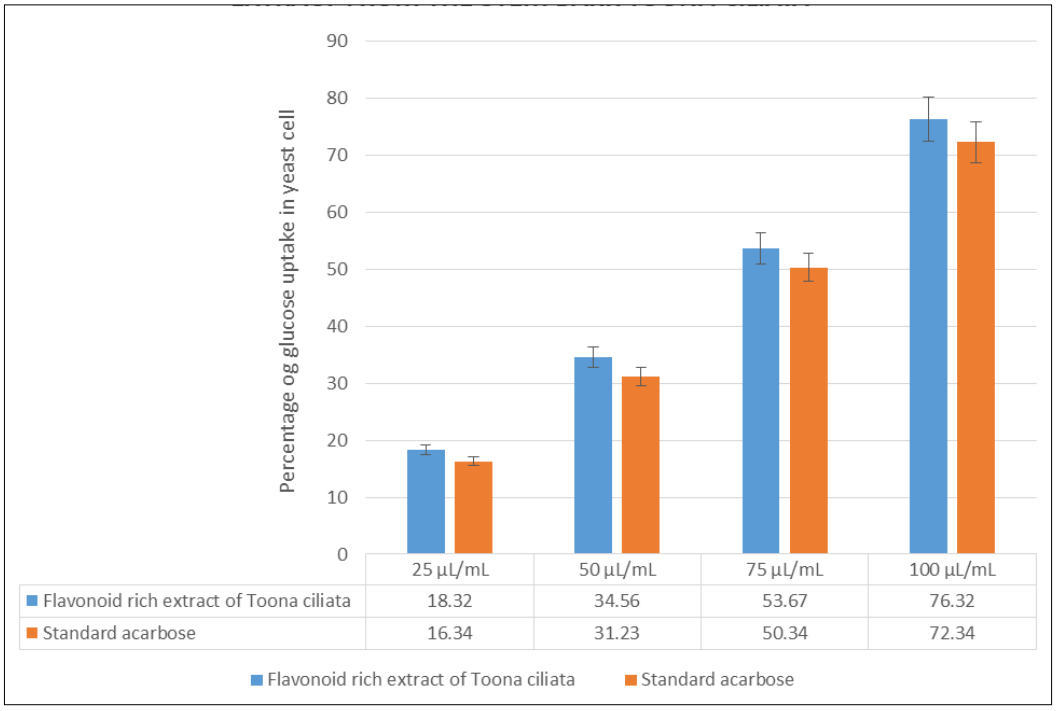


**Fig 1:** Glucose uptake in yeast cells of flavonoid rich extract from the stem bark *Toona ciliata*

**Inhibition of α-Amylase**

In the present study, flavonoid rich extract from the stem bark *Toona ciliata* showed a significant inhibition of αamylase enzyme activity in a concentration dependent manner. Flavonoid rich extract from the stem bark *Toona ciliata* at the concentrations 25, 50, 75 and 100 µg/ml showed 74.45% inhibition of α-amylase enzyme activity, respectively with an $EC_{50}$ value 64.98 µg/ml.

The Glycomet used as a reference standard at the same concentrations showed 71,39% inhibition of α-amylase activity with an $EC_{50}$ value 71.23 µg/ml (Fig-2).

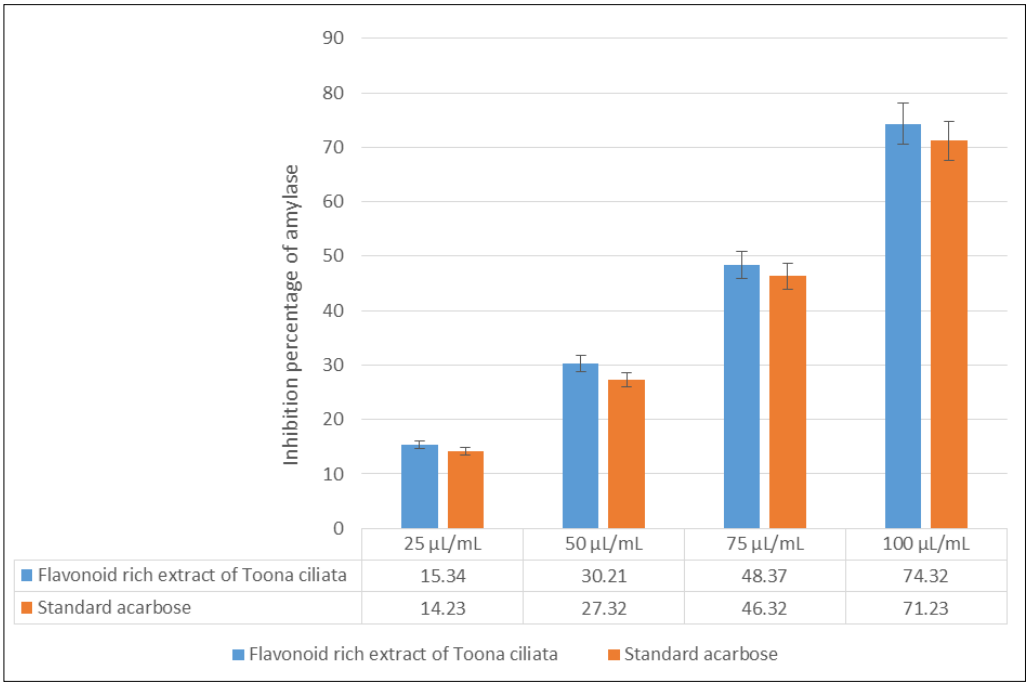


**Fig 2:** Inhibition of α-amylase by flavonoid rich extract from the Stem bark *Toona ciliata*

**Inhibitory activity of α-Glucosidase**

The results of *in-vitro* α-glucosidase inhibitory study are showed in Fig-3. Flavonoid rich extract from the stem bark *Toona ciliata* showed a concentration-dependent inhibition of enzyme. The highest concentration of 100 µl/ml tested showed a maximum inhibition of nearly 73.89% ($EC_{50}$ 66.23µg/ml) in flavonoid rich extract from the stem bark *Toona ciliata* seems to be less potent in α-glucosidase inhibitory potential compared to Glycomet. It may be that α-glucosidase is more sensitive towards glycomet with the concentration required for 50% inhibition ($EC_{50}$) found to be 68.79µg/ml. Apart from that polyphenolic compounds were found in fraction 2, may interact or inhibit specific positions in enzymes thereby reducing the potency of α-amylase and α-glucosidase (Nurhayati *et al*., 2017) [11].

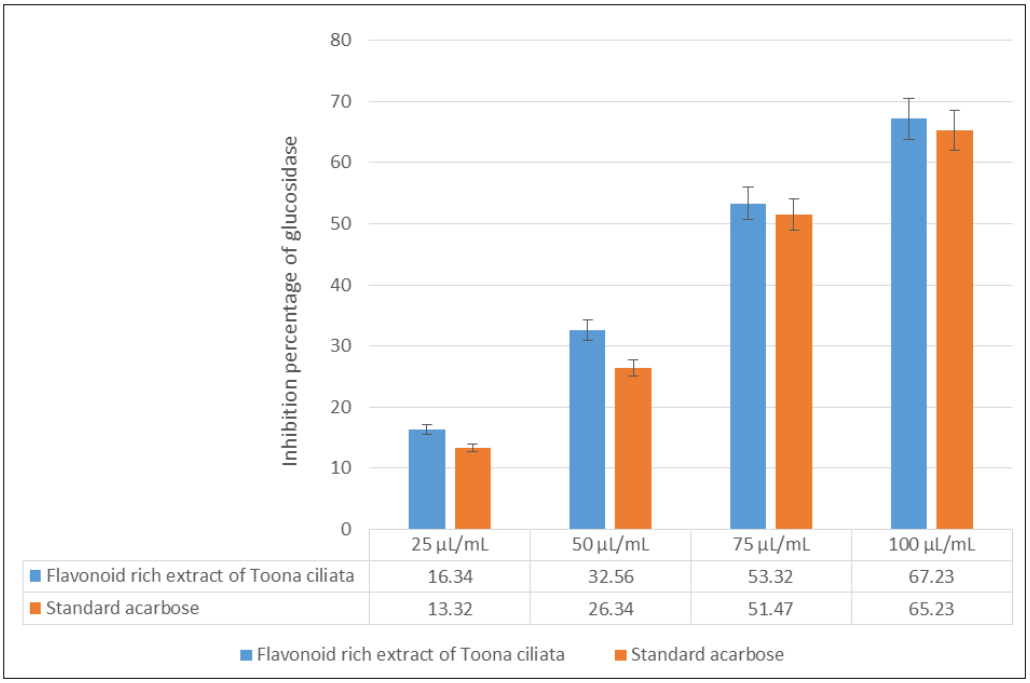


**Fig 3:** Inhibitory activity of α-glucosidase of flavonoid rich extract from the stem bark *Toona ciliata*

**Antibacterial activity**

Antibacterial activity of flavonoid rich extract from the stem bark *Toona ciliata* tested against *Staphylococcus aureus, Escherichia coli* and *Enterococcus faecalis*, *Pseudomonas aeruginosa* were assessed as inhibition zones in the agar plates (Table-2). In this experimental all the bacteria were found to be sensitive to the polyphenol rich fraction. Additionally, the zone of inhibition reconsideration that the polyphenol rich fraction influenced antibacterial activity in proportion to concentration gradient ranges 25-100 µl/ml against the tested bacteria. Amongst the bacteria considered, *Staphylococcus aureus* and *Escherichia coli* was identified to be highly susceptible followed by *Pseudomonas aeruginosa* and *Enterococcus faecalis*. This may confirm the antibacterial property of flavonoid rich extract. Medicinal plants are an important source for the discovery of potential new agents to control pathogens. In the present study, correlated that glabrol, licochalcone A, licochalcone C, and licochalcone E displayed high efficiency against *S. aureus* (Shuai-Cheng *et al*., 2019) [14]. Flavonoid compound such as Licochalcone A and Licochalcone E inhibited the secretion of alpha-toxin enterotoxins A and B by *S. aureus*, which play an important role in pathogenesis (Qiu *et al*., 2010) [12].

**Table 2:** The antibacterial activity of the flavonoid rich extract from the stem bark *Toona ciliata* 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* | 11.3±1.4 | 12.5±0.9 | 15.3±0.7 | 17.2±0.7 |
| *Escherichia coli* | 8.3±2.5 | 10.1±1.3 | 13.4±1.7 | 15.3±1.9 |
| *Pseudomonas aeruginosa* | 7.6±1.4 | 9.3±0.6 | 12.7±2.1 | 14.7±0.9 |
| *Enterococcus faecalis* | 6.8±1.7 | 8.7±1.7 | 10.9±1.7 | 12.6±1.4 |

*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 complete sequences, the MIC of flavonoid rich extract from the stem bark *Toona ciliata* ranged between 25 to 100 µg/ml against gram positive bacteria and gram negative bacteria, (*Staphylococcus aureus, Escherichia coli, Enterococcus faecalis,* and *Pseudomonas aeruginosa*) respectively. The Minimum inhibitory absorption value of flavonoid rich extract from the stem bark *Toona ciliata* increases with increase in concentration. *S. aureus* exhibited maximum inhibition when compared to the other pathogenic bacteria at 100 µl/ml concentration. *Enterococcus faecalis, Escherichia coli* appearances reasonable range of inhibition activity. *P. aeruginosa* display slighter activity. In comparison with gram positive bacteria and gram negative bacteria, the MIC of flavonoid rich extract from the stem bark *Toona ciliata* displayed highest inhibition in gram negative bacteria and among the gram positive bactertia *S. aureus* showed maximum inhibition (Graph-1). The inhibitory effect of peptidoglycan on the antibacterial activity of glabrol supported that flavonoid glabrol first binds to the peptidoglycan of *S. aureus* (Kuhn *et al*., 2015) [9].

**Conclusion**

In conclusion, results specify that the flavonoid rich extract from the stem bark *Toona ciliata* possess antidiabetic and antioxidant properties at varying levels. Flavonoid rich extract from the stem bark *T. ciliata* showed higher antidiabetic, alpha amylase and glucosidase inhibition activity. Pearson's correlation studies showed that there were significant correlations between estimated antidiabetic and antioxidant properties. Results indicate that these antidiabetic and antibacterial activities may be due to the occurrence of bioactive flavonoid compounds in these stem bark of *T. ciliata*.

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