Hypoglycaemic Activities of Bark from *Cassia glauca* Lam.

Ni Ni Aye¹, Nwe Nwe Aung² and May Aye Than³

 Assistant Lecturer, Department of Botany, University of Yangon.
 Assistant Lecturer, Department of Botany, Hta-wei University.
 Deputy Director and Head, Pharmacological Research Division, Department of Medical Research (DMR) (Lower Myanmar)

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ABSTRACT

Cassia glauca Lam. is the medicinal plant belongs to the family Caesalpiniaceae. It is known as "Pyi-ban-nyo" or "Pyi-banshwe" in Myanmar. In antimicrobial test, the various solvent extract of leaves and bark were tested on six pathogenic microorgansims. According to the result, the bark extracts were showed more activity than the leaves extracts of *Cassia glauca* Lam. The acute toxicity of aqueous extract and 70% ethanolic extract from the bark of Cassia glauca Lam. was tested on mice. It was observed that the both extracts were free from harmful effect during observation period of two weeks with maximum permissible dose of 2g/kg. In this research, hypoglycaemic activity of aqueous and 70% ethanolic extracts of Cassia glauca Lam. bark was studied on adrenaline induced hyperglycaemic rats. These effect were also compared with control and standard drug glybenclamide. The effect of aqueous extract was faster than 70% ethanolic extract.

INTRODUCTION

- Myanmar is rich in plants, many of which are of medicinal values.
 Medicinal plants are important for drug development.
- At present, Government of Myanmar has initiated a national programme for the Development of Traditional Medicine System to combat six major types of diseases namely malaria, tuberculosis, diarrhoea, dysentery, hypertension and diabetes mellitus.
- The projected increase of diabetes in Myanmar is estimated by W.H.O to be 16.3% by the year in 2025 (Khin Ye Myint, 2003).
- Myanmar populations have been used many medicinal plants claimed to have hypoglycaemic activity (May Aye Than, 2001).
- The leaves and bark of *Cassia glauca* Lam. were used for diabetes and gonorrhoea (Chopra, 1956).
- The hypoglycaemic activity of bark from *Cassia glauca* Lam. also had not been tested scientifically in Myanmar. Therefore, the antimicrobial activity, acute toxicity and hypoglycaemic activity of this plant had been conducted in this study.

AIMS AND OBJECTIVES

- To determine the antimicrobial activity of leaves and bark from *Cassia glauca* Lam. on six pathogenic microorganisms.
- To study the acute toxicity from the bark of *Cassia glauca* Lam. on albino mice.
- To evaluate the hypoglycaemic effect of 70% ethanolic and aqueous extracts from the bark of *Cassia glauca* Lam.
 on adrenaline-induced hyperglycaemic rats model.

MATERIALS AND METHODS

Antimicrobial Activity Test Materials

Apparatus

Autoclave, hot air sterilizer, water-bath, clean bench, steam-drying oven, test tubes, conical flask, measuring cylinder, micropipettes, pipette tips, petridishes, loop and cotton wool.

Test organisms

The test organisms were used *Bacillus pumalis, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureous* and *Candida albicans* from the Development Center of Pharmaceutical and Food Technology.

Collection

The leaves and barks of *Cassia glauca* Lam, were collected from near the Yangon Technological University, Yangon Division were washed, air dried in room temperature about 15 days.



After that samples were pulverized by grinding with a mortar and pestle and then, packed in bottle and stored at room temperature.

Extraction

The dried powder samples of leaves and bark were extracted with petether, chloroform, ethyl acetate, acetone, ethanol, methanol and aqueous by percolation method.

Methods

The antimicrobial activities were tested by using agar-well diffusion method. Nutrient agar were prepared according to the method describe by Cruickshank, (1975).

Acute Toxicity Test (Materials)



Figure (1)Weighing mice in balance



Figure (3)Administration of drug suspension to the mice



Figure (2)Each group contains 10 mice



Figure (4)Weighing rat in balance

Hypoglycaemic Activity Test (Materials)



Figure (5)Albino rats



Figure (6)Administration of drug suspension to the rat



Figure (7)Adrenaline injection to the rat



Figure (8)Determination of blood glucose 8 level by using glucometer

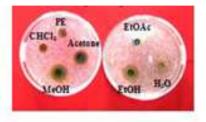
Methods

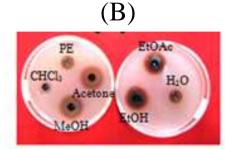
- Acute toxicity test was done by using the method of Litchfield and Wilcoxon (1949).
- Hypoglycaemic activity test was done according to the method of Gupta, *et al.*, (1967)
- Before the drug administration, the blood sample (0.2ml) was collected by cutting about 1mm at the tip of tail as the base line blood sample (0 hour).
- Then, distilled water (10ml/kg) was administered orally just after 0.5 hr administration of distilled water, these rats were subcutaneously injected with 0.8 mg/kg body weight of adrenaline tartrate.
- Then, blood glucose levels were taken at hourly to 4 hrs.
- After drug free interval of one week, these rats were orally given with 70% ethanolic extract, aqueous extract and glibenclamide.
- The blood glucose levels were determined as in above procedure.

RESULTS

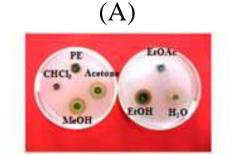
• In this experiment, the bark extracts were showed highest activity on all six microorganisms.

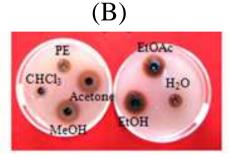
(A)





Bacillus pumalis





Staphylococcus aureus





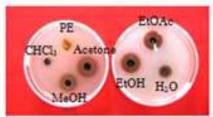
Bacillus subtilis



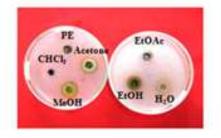


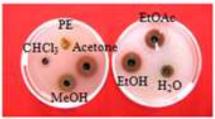
Pseudomonas aeruginosa





Candida albicans





Escherichia coli

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- The acute toxicity test was observed that aqueous and 70% ethanolic extracts showed lethality effect.
- Aqueous extract was observed that the mice die within 2 days after oral administration of aqueous extract at the dose levels of 4 g/kg, 6 g/kg and 8 g/kg body weight.
- The 70% ethanolic extract was observed that the mice die within 24 hours after oral administration of ethanolic extract at the dose levels of 4 g/kg, 6 g/kg and 8 g/kg body weight.
- At the minimum dose of 2 g/kg body weight of aqueous and 70% ethanolic extracts, there was no lethality.
- The hypoglycaemic effect of aqueous and 70% ethanolic extracts of bark of *Cassia glauca* Lam. were tested by using adrenaline induced hyperglycaemic albino rats.

• The results of hypoglycaemic activity were shown in figure.

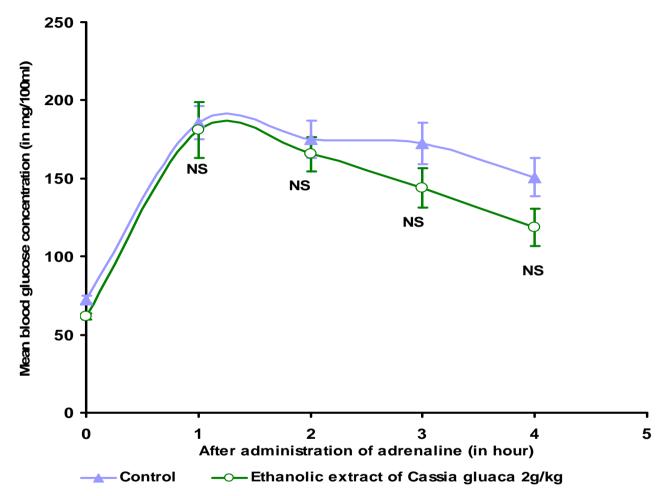


Figure 10. Time course effect of 70% ethanolic extract from bark of *Cassia* glauca Lam. on adrenaline induced hyperglycaemic rat model Each point represents the mean of observations and the vertical bars indicate standard errors of the means.

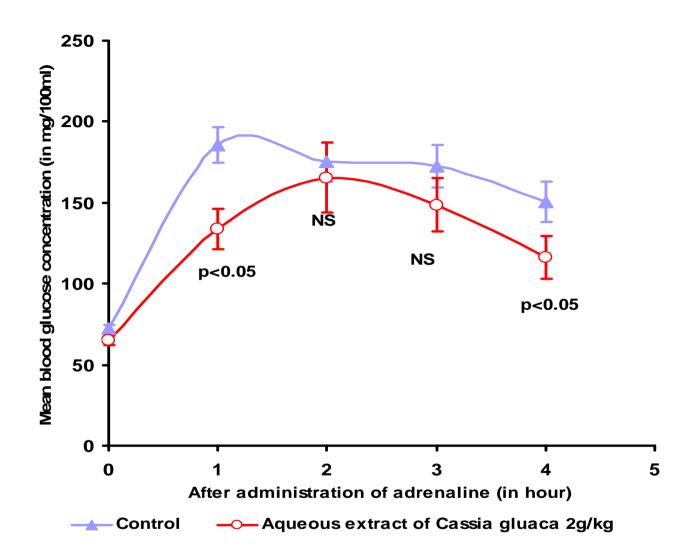


Figure 11. Time course effect of aqueous extract from bark of Cassia glaucaLam. on adrenaline induced hyperglycaemic rats modelEach point represents the mean of observations and the verticalbars indicate standard errors of the means.13

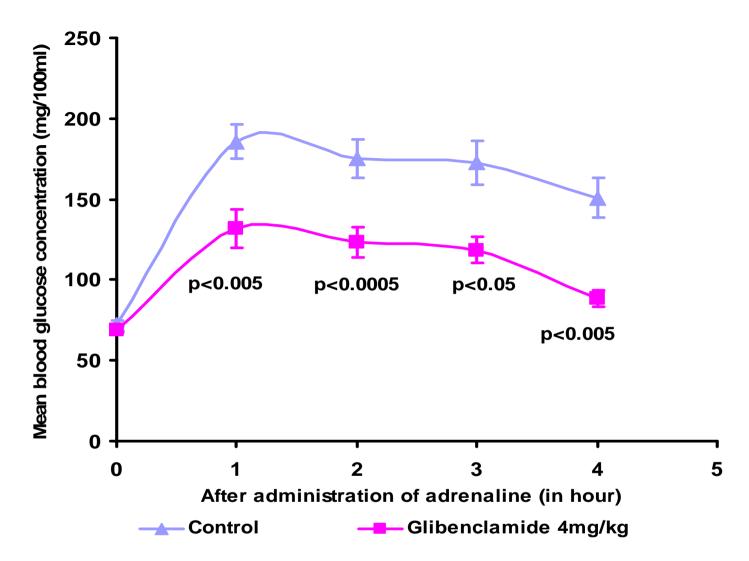


Figure 12. Time course effect of standard drug glibenclamide on adrenaline induced hyperglycaemic rat model Each point represents the mean of observations and the vertical bars indicate standard errors of the means.¹⁴

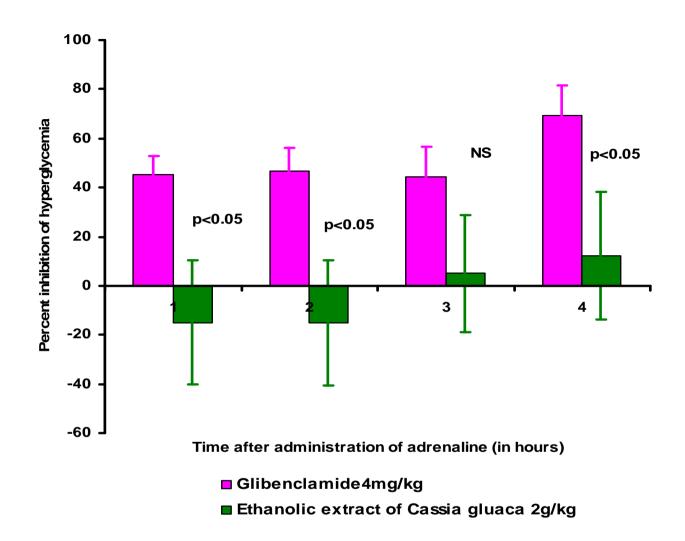
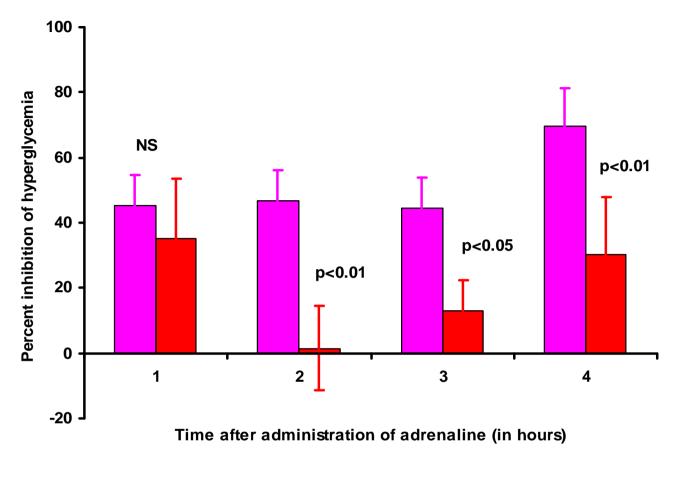


Figure 13. Comparative percent inhibition effect of 70% ethanolic extract from bark of *Cassia glauca* Lam. and glibenclamide on adrenaline induced hyperglycaemic rat model Each point represents the mean of observations and the vertical bars indicate standard errors of the means.



■ Glibenclamide4mg/kg ■ Aqueous extract of Cassia gluaca 2g/kg

Figure 14. Comparative percent inhibition effect of aqueous extract from bark of *Cassia glauca* Lam. and glibenclamide on adrenaline induced hyperglycaemic rat model Each point represents the mean of observations and the vertical bars indicate standard errors of the means.

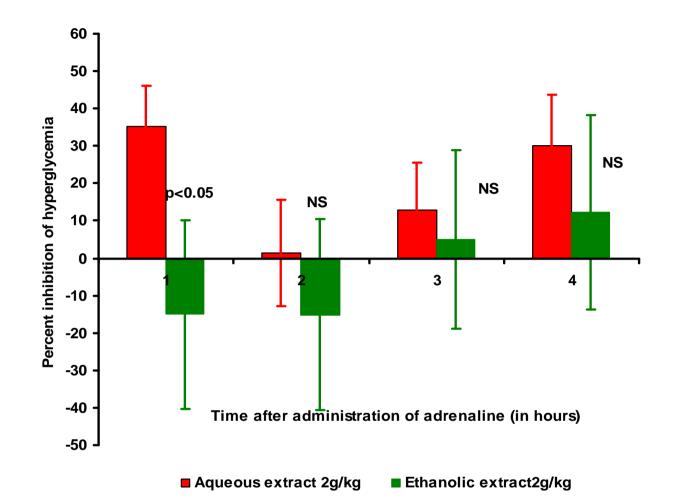


Figure 15. Comparative percent inhibition effect of 70% ethanolic extract and aqueous extract from bark of *Cassia glauca* Lam. on adrenaline induced hyperglycaemic rat model Each point represents the mean of observations and the vertical bars indicate standard errors of the means.

DISCUSSION AND CONCLUSION

- In this research, pharmacological aspects such as antimicrobial activity, acute toxicity and hypolycaemic activity of *Cassia glauca* Lam. have been conducted.
- In antimicrobial activity, the bark extracts were showed more activity than the leaves extracts.
- The acute toxicity of 70% ethanolic and aqueous extracts from bark of *Cassia glauca* Lam. which showed lethality on albino mice.
- The medium lethal dose (LD₅₀) of aqueous extract and 70% ethanolic extract 9 g/kg (6.67-12.15 g/kg confidence limit) and 11.7 g/kg (confidence limit was 8.07-16.97) respectively.
- Gosh (1984) reported that the LD₅₀ of 7 g/kg body weight to 21 g/kg body weight on mice was practically non-toxic.

- Therefore, the results of this study was observed between practically non-toxic dose level and the acute toxicity of aqueous and 70% ethanolic extracts were practically non-toxic.
- In this research, the hypoglycaemic effect of the 70% ethanolic extract was not significantly at 1hr up to 4hr, the aqueous extract was significant at 1hr and 4hr while the standard drug glibenclamide was observed that significantly at 1hr, 2hr, 3hr and 4hr, when compared with that of control group.
- The percent inhibition effects of aqueous extract and glybenclamide were not significantly at 1hr, while the 70% ethanolic extract was significantly lowered at 1 hr and 2 hr when compared with glibenclamide.
- These findings suggest that the action of aqueous extract was more effective than 70% ethanolic extract.
- Therefore, the aqueous extract of bark of *Cassia glauca* Lam.
 possess hypoglycaemic effect on adrenaline induced hyperglycaemic rat model.

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REFERENCES

Chopra, R. N. 1956. Glossary of Indian Medicinal Plants, Council of Scientific and Industrial Research, New Delhi.
Cruickshank, R., Duguid, J.P., Marmion B. P., Swain R. H. A. 1975. Medical Microbiology, 12th ed., (Vol.2), Printed in Great Britain, Distributed in the USA by Longman Inc. New York.

Diabetes Project, 1998. **Prevention and Control of Diabetes Mellitus.** Handbook for doctors and nurses. Ministry of Health, Myanmar.

Ghosh, M. N., 1984. **Fundamentals of experimental Pharmacology**, 2nd Edition, Scientific Book Agency, Calcutta. Gupta, S. S., S. C. L., Verma, V. P. Garg, and M. Ri. 1967. Antidiabetics effect of *Tinospora cordifolia*. Indian Journal of Medicinal Research, 545 (7), 733-745.

- Khin Ye Myint. 2003. **Statistic in Diabetics**. In : Proceedings of Panel on Diabetes : Global Epidemic and Myanmar Academy of Medicinal Science, Yangon. pp. 3-8.
- Litchfield, J. T and F. A. Wilcoxon. 1949. A simplified method of evaluating dose effect experiments. Journal of Pharmacology and Experimental Therapeutic, 96, 99-113. Standard Research Laboratories. Stanford : American Cyamamid Company.
- May Aye Than. 2001. **Traditional Medicine Research in Department of Medical Research** (Lower Myanmar) DMR Bulletin, 15 : 1-2.
- World Health Organization. 1999. **Medicinal Plants in China**. Regional Office for the Western Pacific, Manila.

