Journal of Applied Pharmaceutical Research
Volume 6, Issue 3, Year of Publication 2018, Page 01 – 06
DOI: 10.18231/2348-0335.2018.0008

Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR
www.japtronline.com *ISSN: 2348 – 0335*

ASSESSMENT OF CALAMUS TENUIS FRUITS EXTRACT ON BLOOD GLUCOSE LEVEL ELEVATION AND ITS ANTIBACTERIAL POTENCY

Hema Sarkar[1], Tasfia Sharin[2], Muhammad Shamim Al Mamun[1*]

Article Information
Received: 19th June 2018
Revised: 17th September 2018
Accepted: 22nd September 2018

Keywords
Antibacterial; Blood glucose level; Extraction; Calamus tenuis; positive control

ABSTRACT
Investigation of anti-hyperglycemic and antibacterial activity of the fruit extract of *Calamus tenuis* was carried out. Soxhlet extraction was done using petroleum ether, ethyl acetate and methanol. Anti-hyperglycemic activity was done by Oral Glucose Tolerance Test.The results indicated that methanol extract (500 mg/Kg) showed nearly same potency of blood glucose lowering capacity (4.82 ± 0.730 mmol/L) compared to the glibenclamide (standard drug). Antibacterial activity was investigated using disc diffusion method. Antibacterial tests revealed that *S. aureus* was resistant to all the solvent extracts. Ethyl acetate and methanol extracts showed moderate antibacterial capacity against both *Micrococcus* and *V. cholerea* whereas petroleum ether showed nothing. Zone of inhibition of Ethyl acetate and methanol extracts (200 mg/mL dose) for *E. coli* were very close to positive control (17.00 ± 0.816 and 18.75 ± 1.258 mm respectively; positive control value was 19.00 ± 1.414 and 21.00 ± 1.414 respectively). In case of methanol zone of inhibitions were greater than other solvent extracts against all tested bacterial species. From the present research it can be recapitulated that due to the biological activity of *C. tenuis* fruit it may satisfy as a new candidate for the emergence of medicines.

INTRODUCTION

It is documented from World's Health Organization that 80-85% of populations are depending on medicinal plant for any types of diseases. Thousands of medicinal plants have been used from hundreds of years ago and *Calamus tenuis* is one of them [1-3]. Rattan, which scientific name is *Calamus tenuis*, is known as Bet in Bangladesh. In South-East Asian countries *Calamus tenuis* fruits, tender shoots have been consumed as significant medicine and dietary alternative due to their proteins, carbohydrates, minerals, fiber content and remedial action against different types of illness [4-6]. Many researchers had reported on *Calamus tenuis* fruits which evaluate the phytochemical potential, antioxidant and cytotoxic activity, analgesic and central nervous system depressant activities [7,

[1]*Chemistry Discipline, Khulna University, Khulna – 9208, Bangladesh*
[2]*Applied Nutrition and Food Technology, Islamic University, Kushtia, Bangladesh*

***For Correspondence:** smamun45@gmail.com*

8]. Locally, as traditional medicine, *Calamus tenuis* is used for treating fever, piles, bacterial infection [9], inflammation [10], diabetic [11], intestinal worm infection and stomach disorder [12].

This inventory will be the report on antibacterial activity and anti-hyperglycemic activity of *Calamus tenuis* fruits. If the results imply towards these potential of this fruits, it will justify its occasional consumption as a functional food.

MATERIALS & METHODS

Chemicals

All solvents and chemicals used were of analytical grade

Sample Collection

Places near to barui para village at Magura union in Satkhira district were selected for the collection of the fruits of *Calamus tenuis*. Collection was done during the month of June 2017.

Extraction

Collected sample was cleaned, broken into tiny sizes and then shade dried for 15 days. Soxhlet extraction was carried out using three different solvents (petroleum ether, ethyl acetate and methanol, each of 200 mL). The temperature was maintained at 45°C and extraction was continued for 10 hours. The extract is filtered. Excess solvent is removed using a rotary vacuum evaporator.

Animal used

Approximately indifferent weight of 20-30 g of 4-5 weeks old white albino mice were purchased from Jahangirnagar University, Bangladesh and kept them 12 h light and 12 h dark cycle in standard condition in the animal house [13]. Formulated mice food and water were supplied to the mice. Cages were cleaned on every day. They were adapted to laboratory condition for a week before the experiment.

Anti-hyperglycemic Activity Test

This activity was done in light of the previous experiment [13]. In briefly, mice was segmented to control, reference and test group. Test samples, standard and control solution were administered orally using a feeding needle according to the corresponding dose. After 30 min glucose solution (2 g/kg body weight) was given to the mice. Blood glucose level was estimated at 0, 60 and 120 min after glucose administration using a glucometer and blood glucose strips.

Antibacterial Activity Test

Two types of bacteria were used for the antibacterial activity test. All the experiments were performed as described by the literature [14]. Shortly, antibacterial test was carried out by disc diffusion method. To maintain the sterility laminar flow cabinet was used. For the growth of bacteria nutrient agar media was exercised. Whitman No 1 filter paper discs of 6mm diameters were saturated into the stock solution of the complexes (10–25 mg/ml) and dried under sterile conditions. The dried discs were then placed on the previously vaccinated agar surface. The plates were incubated for 24 hours at 30°C. Antimicrobial activity was revealed by the presence of clear inhibition zones around the discs.

Statistical Analysis

Anti-hyperglycemic analysis was carried out five times for each test and presented as average ± standard deviation (SD) whereas antibacterial analysis was done four times for each test and expressed as average ± standard deviation (SD). Data were analyzed using Microsoft Excel 2007. Dixon's Q test was run for the marking off and refusal of outliers. With five observations of anti-hyperglycemic test and with four observations of antibacterial test and at 95% confidence level, it was observed that all the data were statistically appropriate with no meaningful distinction within the values.

RESULTS & DISCUSSIONS

In this study, in vitro anti-hyperglycemic activity of methanol extract and antibacterial potency of three different extracts of *Calamus tenuis* fruits were evaluated.

Anti-hyperglycemic Activity

Table 1 shows the administered dose variation with the body weight of mice. It represents that standard drug and extracts needed first hour to be active for lowering blood glucose level. After that there was a gradual declination of blood glucose levels from the first hour to the end of the test. This scenario could be verified by the group [15].

Figure 1 represented that maximum anti-hyperglycemic activity (4.82 ± 0.730 mmol/L) of methanol extract was observed with a 500 mg/kg dose after 120 minutes of glucose loading which was very close to the positive control (4.26 ± 0.441 mmol/L) whereas in case of 250 mg/kg dose it was 6.26 ± 0.307 mmol/L, which represented the moderate glucose lowering

capacity compared to the standard drug. This study alluded that the methanol extract of *C. tenuis* fruits has significant glucose lowering capacity at all doses in a dose dependent manner due to avail the polyphenols such as flavonoids and anthocyanins [16].

Dose dependent significant blood glucose lowering potency at different time interval of *Calamus tenuis* fruits extracts compared to the standard glibenclamide, evaluated by the GTT was verified by the literature [17].

Table 1. Impact of *C. tenuis* fruits methanolic extract on blood glucose level.

Samples	Number of mice	Weight of mice (gm)	Dose(mL)	Blood Glucose Level (mmol/L)		
				0 min	60 min	120 min
Control	1	27	0.27	5.5	6.9	6.1
	2	30	0.30	6.8	7.7	6.9
	3	31	0.31	7.1	9.7	8.1
	4	23	0.23	7.5	10.5	10.1
	5	25	0.25	7.0	10.7	9.5
Positive control Glibenclamide (5 mg/kg)	1	33	0.33	5.2	6.4	4.8
	2	30	0.30	4.9	6.0	4.5
	3	35	0.35	4.7	6.3	4.4
	4	26	0.26	4.9	5.7	4.1
	5	26	0.26	5.2	5.8	3.5
Extract (250 mg/kg)	1	27	0.27	5.9	8.5	6.6
	2	32	0.32	5.1	7.8	6.5
	3	30	0.30	6.7	7.5	6.4
	4	32	0.32	6.3	6.9	6.0
	5	31	0.31	7.2	6.5	5.8
Extract (500 mg/kg)	1	29	0.29	5.1	6.8	4.3
	2	30	0.30	5.5	6.1	3.9
	3	33	0.33	6.4	6.9	4.7
	4	33	0.33	5.2	7.8	5.2
	5	32	0.32	7.1	8.0	6.0

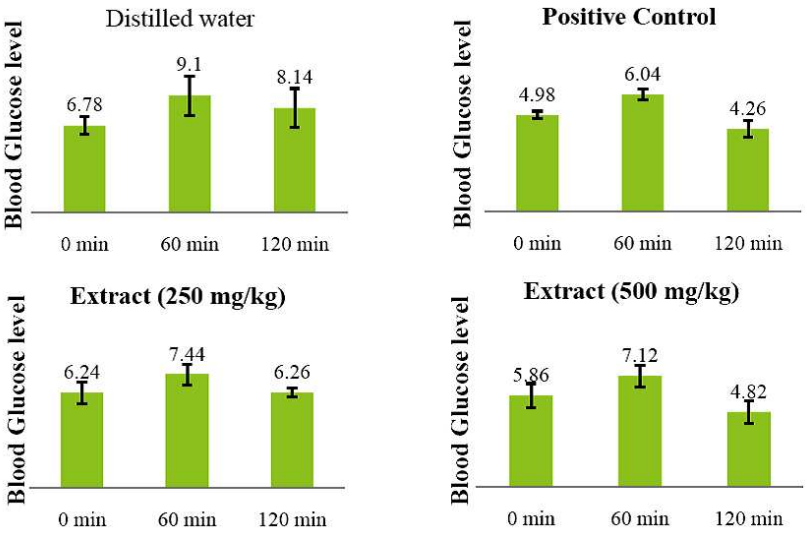

Fig. 1: Outcome of methanolic extract (250 mg/kg and 500 mg/kg body weight), control (distilled water), standard (glibenclamide) on mice blood glucose level

Table 2. Inhibition zone of petroleum ether, Ethyl acetate and Methanol extract of *C. tenuis* fruit

Fruit Extract	Bacterial species	Zone of inhibition (mm) [Average ± Standard Deviation (SD)]		
		Test sample (100 mg/mL)	Test sample (200 mg/mL)	Positive control (10 mg/disc)
Petroleum Ether Extract	*S. aureus*	0.00 ± 0.000	0.00 ± 0.000	11.00 ± 1.414
	Micrococcus	0.00 ± 0.000	0.00 ± 0.000	18.00 ± 2.828
	V. cholerea	0.00 ± 0.000	0.00 ± 0.000	9.50 ± 0.707
	S. typhi	7.00 ± 0.816	8 .00 ± 1.414	13.00 ± 1.414
	E. coli	7.25 ± 0.957	10.75 ± 2.986	22.00 ± 2.828
Ethyl Acetate Extract	*S. aureus*	0.00 ± 0.000	0.00 ± 0.000	19.00 ± 1.414
	Micrococcus	6.00 ± 0.816	6.00 ± 0.816	18.00 ± 2.828
	V. cholerea	7.75 ± 0.957	8.50 ± 1.000	12.50 ± 0.707
	S. typhi	7.25 ± 0.957	8.25 ± 0.957	19.00 ± 1.414
	E. coli	11.25 ± 2.217	17.00 ± 0.816	19.00 ± 1.414
Methanol Extract	*S. aureus*	0.00 ± 0.000	0.00 ± 0.000	11.00 ± 1.414
	Micrococcus	8.25 ± 1.258	9.25 ± 0.957	19.00 ± 1.414
	V. cholerea	8.25 ± 0.957	9.25 ± 0.500	15.50 ± 0.707
	S. typhi	7.75 ± 1.258	8.50 ± 0.577	19.00 ± 1.414
	E. coli	18.00 ± 1.633	18.75 ± 1.258	21.00 ± 1.414

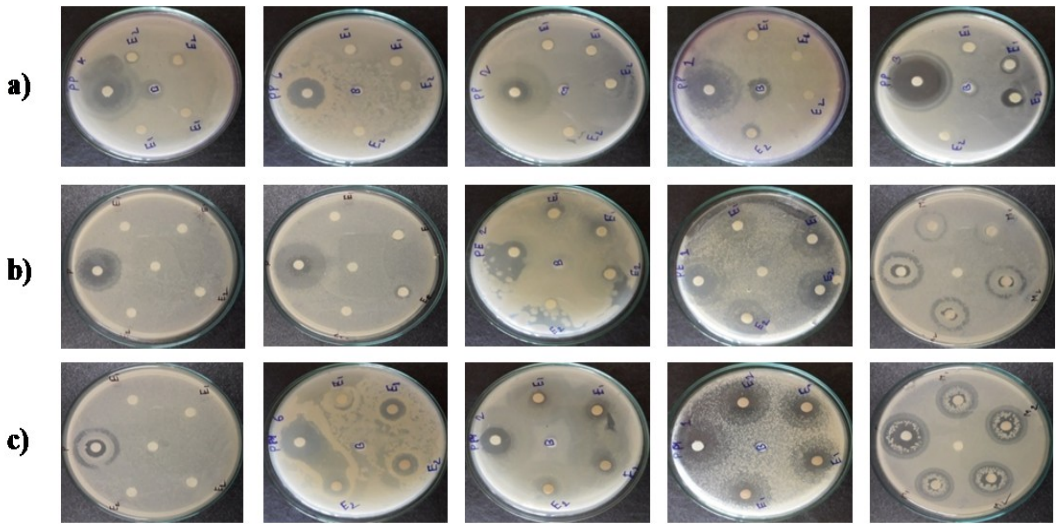

Figure 2. Photogenic representation of zone of inhibition of a) petroleum ether, b) ethyl acetate and c) methanol extract against microorganisms (from left to right sequence: *S. aureus, Micrococcus, V. cholerea, S. typhi* and *E. coli*).

Antibacterial Activity

Figure 2 displays the photogenic representation of zone of inhibition of all the extracts against the tested bacterial species in this study. Table 2 displays that every extract applied for the antibacterial activity was active against at least 2 among 5 bacterial species. According to previous study, plant species used for therapeutic treatments inhibited the growth of *Staphylococus aureus*, *Escherichia coli* and *Salmonella typhi* [18, 19].

In figure 3, it is clearly seen , the petroleum ether extract showed maximum activity against *E. coli* (10.75 ± 2.986 mm)and moderate activity against *S. typhi* (8 .00 ± 1.414 mm) for 200 mg/mL extract compared to the positive control.*S. aureus* was resistant to all the tested solvent extracts. Ethyl

acetate and methanol extracts showed higher activity than petroleum ether against the pathogens. *Micrococcus* represented identical activity (6.00 ± 0.816 mm) for 100 mg/mL and 200 mg/ mL ethyl acetate extract.

This extract exhibited maximum inhibition on *E. coli* (17.00 ± 0.816 mm) which was near to positive control (19.00 ± 1.414). Methanol extract displayed almost same activity against *E. coli* (18.75 ± 1.258 and 18.00 ± 1.633 mm) for 200 mg/mL and 100 mg/mL dose concentration respectively. These findings could be compared with the literatures [18-21], in which it was said methanolic extract showed high antimicrobial activity because of some special compounds.

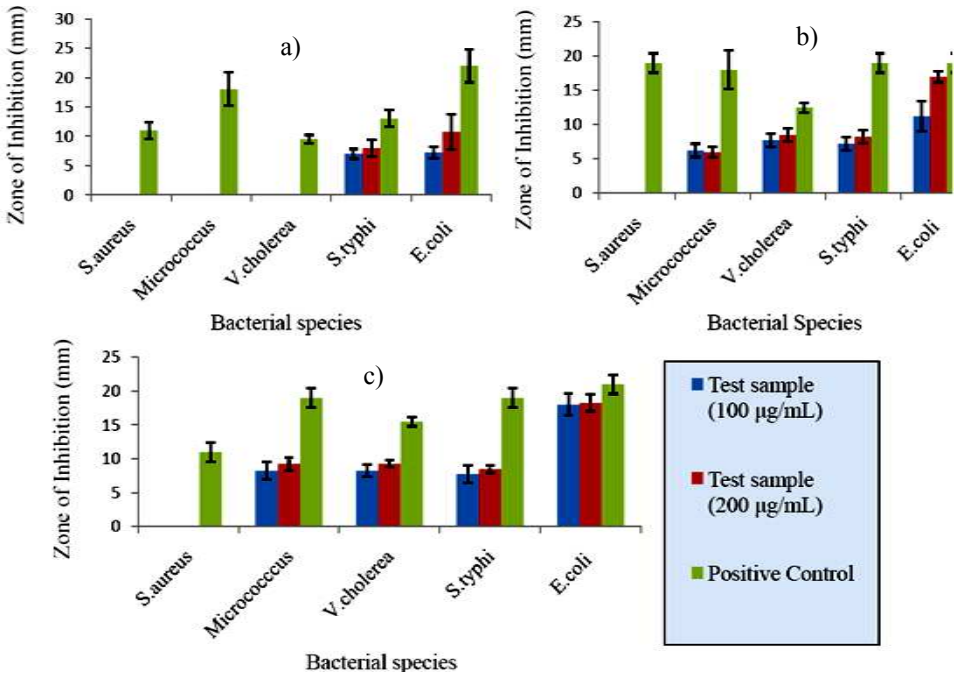

Fig 3. Inhibition zone of a) petroleum ether, b) ethyl acetate and c) methanol extracts against microorganisms

CONCLUSION

C. tenuis fruits extracts are the most effective plant extracts against the Gram-negative bacteria studied in this work. *C. tenuis* fruits exert promising anti-hyperglycemic effects in mice. The anti-hyperglycemic agents can be explored for the development of anti-diabetic molecules.

ACKNOWLEDGMENT

The authors acknowledge Chemistry Discipline for financial support and Pharmacy Discipline and Biotechnology and Genetic Engineering Discipline of Khulna University for the facilities of this research work.

FINANCIAL ASSISTANCE

This research was funded by Research Cell, Khulna University, Khulna-9208, Bangladesh. File no-KU/Research Cell/04/2000-41

CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCES

[1] Ignacimuthu S, Ayyanar M, Sivaraman SK. Ethnobotanical investigations among tribes in Madurai district of Tamil Nadu (India). *Journal of Ethnobiology and Ethnomedicine*, 2, 1-7 (2006).

[2] Elujoba AA, Odeleye OM, Ogunyemi CM. Traditional medicine development for medical and dental primary health care delivery system in Africa. *African Journal of Traditional, Complementary and Alternative Medicine*, 2, 46-61 (2005).

[3] Tomlinson TR, Akerele O. Medicinal plants: their role in health and biodiversity. *University of Pennsylvania Press, Philadelphia*,11-41 (1998).

[4] Durst PB, Ulrich W, Kashio M. Non-wood forest products in Asia. *Bangkok; Rapa Publication* (1994).

[5] Manohara TN. Nutritional Evaluation of Shoots of Two Rattans of Northeast India- *Calamus flagellum* Griff. Ex Mart. and *C. floribundus* Griff. (Arecaceae). *Econ Bot*, 67(3), 263–268 (2013).

[6] Dransfield J, Tesoro FO, Manokaran N. Rattan: current research issues and prospects for conservation and sustainable development. *Rome; FAO* (2002).

[7] Ahmed ZU, Bithi SS, Khan MR, Hossain M, Sharmin S, Rony SR. Phytochemical screening, antioxidant and cytotoxic activity of fruit extracts of *Calamus tenuis Roxb*. *J Coastal Life Med,* 2(8), 645-650 (2014).

[8] Hossain MS. Analgesic and Neuropharmacological activity of methanolic extract of *Calamus tenuis* Roxb. Fruits. *J Sci Innovative Res*, 2(6), 1067-1072 (2013).

[9] Khare CP. Indian medicinal plants: an illustrated dictionary. *Springer Science & Business Media*(2007).

[10] Yu GF, Mulabagal V, Diyabalanage T, Hurtada WA , DeWitt DL, Nair MG. Nonnutritive functional agents in rattan-shoots, a food consumed by native people in the Philippines. *Food Chem*, 110, 991–996 (2008).

[11] Tag H, Kalita P, Dwivedi P, Das AK, Namsa ND. Herbal medicines used in the treatment of diabetes mellitus in Arunachal Himalaya, northeast, India. *J Ethnophmacol*, 141(3), 786-95 (2012).

[12] Saikia P, Khan ML. Diversity of Medicinal Plants and their uses in home gardens of upper Assam, Northeast India. *Asian J Pharm Biol Res*, 1(3), 296-309 (2011).

[13] Joy KL, Kuttan R. Anti-diabetic activity of *Picrorrhizakurroa* extract. *J Ethnopharmacol*, 67(2), 143-48 (1999).

[14] Bauer AW, Kirby WM, Sherries JC, Truck M. Antibacterial susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45, 493-496 (1966).

[15] Eddouks M, Maghrani M. Effect of *Lepidium sativum* L. on Renal Glucose Reabsorption and Urinary TGF-β1Levels in Diabetic Rats. *Phytother. Res*, 22, 1–5 (2008).

[16] Dalu D, Duggirala S, Akarapu S. Anti-hyperglycemic and hypolipidemic activity of *leeaindica*. *Int. J. Bioassays*, 3 (07), 3155-3159 (2014).

[17] Ghosh A, Banik S, Amin MN, Ahmed J. Evaluation of antinociceptive, antihyperglycemic, and membrane stabilizing activities of *Garcinia lancifolia* Roxb. *Journal of Traditional and Complementary Medicine xxx,* 1-5(2017).

[18] Maniyar Y, Bhixavatimath P, Agashikar NV. Antidiarrheal activity of flowers of *Ixora coccinea* linn. in rats. *J Ayurveda Integr Med*, 1, 287-291 (2010).

[19] Muroi, H, Kubo I. Antibacterial activity of anacardic acids and totarol, alone and in combination with methicillin, against methicillin-resistant *Staphylococcus aureus*. *J. Appl. Bacteriol*, 80, 387-394 (1996).

[20] Halvorsen BL, Carlsen MH, Phillips KM, Bohn SK, Holte K, Jacobs DR Jr, Blomhoff R. Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. *Am J ClinNutr*, 84, 95-135 (2006).

[21] Chopra PKPG, Saraf BD, Inam S,Deo SS. Antimicrobial and antioxidant activities of methanol extract roots of *Glycyrrhiza glabra* and HPLC analysis. *Int. J. Pharm. Pharm*. *Sci.*, 5(2), 157-160 (2013)