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Comparative study of hypoglycemic and antibacterial activity of organic extracts of four Bangladeshi plants

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ABSTRACT

Objective: To examine hypoglycemic and antibacterial activity against some Gram-positive and Gram-negative bacteria of organic extracts of four Bangladeshi plants.

Methods: An *in vivo* hypoglycemic effect on mice model was used to check the hypoglycemic effect of four Bangladeshi herbal organic extracts viz., roots of *Curculigo recurvata* W. T. Aiton (Satipata) (*C. recurvata*), leaf of *Amorphophallus bulbifer* Roxb. (Olkachu) (*A. bulbifer*), whole plant of *Thunbergia grandiflora* Roxb. (Nillata) (*T. grandiflora*) and leaf of *Stuednera colocasiifolia* K. Koch (Yunnan) (*S. colocasiifolia*) using glibenclamide as a positive control and water as a negative control. They were also tested for antibacterial activity on three Gram-positive and four Gram-negative bacteria by disk diffusion method. *C. recurvata*, *A. bulbifer* and *T. grandiflora* were extracted with methanol and *S. colocasiifolia* was extracted with ethanol.

Results: Among all the plant extract, only ethanol extract of *S. colocasiifolia* leaves at 800 mg/kg dose significantly ($P < 0.01$) reduced fasting glucose level in normal mice as compared to standard drug glibenclamide (5 mg/kg). Ethanol extract of *S. colocasiifolia* leaves at 800 mg/kg dose decreased 20.28% of blood glucose level after 2 h of administration in normal mice, where glibenclamide decreased 39.63%. Methanol extract of *T. grandiflora* didn't show any zone of inhibition against the tested bacteria, but other three extracts showed a wide range of zone of inhibition. However, none of the extract showed antibacterial activity against all the tested bacteria. Methanol extract of *C. recurvata* showed maximum zone of inhibition against *Bacillus cereus* [(10.50 ± 0.50) mm], *Salmonella typhi* [(16.20 ± 1.26) mm], *Escherichia coli* [(13.00 ± 1.00) mm] and ethanol extract of *S. colocasiifolia* showed maximum zone of inhibition against *Staphylococcus aureus* [(11.20 ± 1.26) mm], *Bacillus subtilis* [(12.00 ± 0.50) mm], *Salmonella paratyphi* [(10.80 ± 0.29) mm]. Only methanol extract of *A. bulbifer* showed (8.50 ± 0.50) mm and (7.20 ± 0.76) mm zone of inhibition against *Pseudomonas aeruginosa* at 1000 and 800 µg/disk dose respectively.

Conclusions: Through our study, it was found that *S. colocasiifolia* could be considered as very promising and beneficial hypoglycemic agent. Although *C. recurvata* and *S. colocasiifolia* showed comparable high antibacterial activity, further studies should be needed to develop new antibacterial agent from them. *S. colocasiifolia* may be a potential source for the development of new oral hypoglycemic agent.

1. Introduction

Diabetes mellitus comprises of a gathering of disorders described

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All animal experiments were carried out according to the guidelines of Institutional Animals Ethics Committee (IAEC) and study protocols were approved by the Department of Pharmacy, International Islamic University Chittagong Medical Ethics, Biosafety.

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by hyperglycemia, altered digestion system of lipids, carbohydrates, and proteins and an enlarged danger of complications from vascular disease[1]. Expanded thirst, increased urinary yield, ketonemia and ketonuria are the basic side effects of diabetes mellitus, which occur due to the abnormalities in carbohydrate, fat and protein metabolism. At the point, when ketones body is available in the blood and urine, it is called ketoacidosis. Henceforth, legitimate treatment ought to be taken quickly, otherwise it can prompt other diabetic complications[2]. Diabetes mellitus has brought about critical grimness and mortality because of microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart assault, stroke and fringe vascular sickness) complexities[3]. Apart from, as of now, accessible remedial alternatives for diabetes like oral hypoglycemic agents and insulin, which have restrictions

of their own, many herbal medicines have been suggested for the treatment of diabetes[4]. A variety of phytoconstituents present in medicinal plants are thought to be active on a variety of targets by various modes and mechanisms. They have the potential to impart therapeutic effect in complicated disorders like diabetes and its complications[5].

The discovery of antimicrobials like penicillin from *Penicillium notatum* and various other antibiotics have been initiated for the search for naturally available bioactive molecules from living organisms[6]. Many of the bioactive molecules are secondary metabolites, generated in response to external pressures such as competition for space and potential predators. According to the World Health Organisation[7], 65% of the world's population have incorporated ethnomedicine in their primary health care practice. In some African and Asian countries, 80% of the population depend on traditional medicine for primary health care and about 70% of population in the developed world have used alternative or complementary medicines[7]. For example, of the plants used to treat microbial infections, an estimated (6%) has been screened for specific antimicrobial activities and only a small proportion of these have been studied phytochemicals to identify the active constituents and/or blends[8,9]. So, if a plant found with great antibacterial activity and no resistant to bacteria, it could be great finding.

Curculigo recurvata W. T. Aiton (Satipata) (*C. recurvata*), *Amorphophallus bulbifer* (Roxb.) Bl. (Olkachu) (*A. bulbifer*), *Thunbergia grandiflora* Roxb. (Nillata) (*T. grandiflora*) and *Stuednera colocasiifolia* K. Koch (Yunnan) (*S. colocasiifolia*) are native to Bangladesh. They are used as traditional medicines for cardiac diseases and blood purification. *C. recurvata* is traditionally used as antidote to stop bleeding and roots of this plant have anthelmintic activity[10]. Whole plant of *A. bulbifer* is used as anti-inflammatory and analgesic (tuber of this plant has antibacterial activity and leaves of this plant have anthelmintic activity)[10,11]. Leaves and stem of *T. grandiflora* are used as a poultice in stomach complaints and to treat eye diseases in Chittagong Hill Tracts and it has anthelmintic activity[10]. *S. colocasiifolia* (family: Araceae) is an evergreen herb, which is locally used to treat injuries, cuts, snake and insect bites, and skin ulcers. Whole plant extract of *S. colocasiifolia* has antiarthritic and membrane stabilizing activities[12].

The plant kingdom comprises many species of plants containing substances of medicinal value, which are yet to be explored. A large number of plants are constantly being screened for their possible medicinal value[13]. So the aim of the present studies is to evaluate the hypoglycemic and antibacterial activities of organic extracts of root of *C. recurvata*, leaf of *A. bulbifer*, whole plant of *T. grandiflora* and leaf of *S. colocasiifolia*.

2. Materials and methods

2.1. Plant material

Root of *C. recurvata*, leaf of *A. bulbifer*, whole plant of *T. grandiflora* and leaf of *S. colocasiifolia* were collected from different parts of Chittagong region, Bangladesh. The plants were identified by Dr. Shaikh Bokhtear Uddin, Taxonomist and Associate Professor, Department of Botany, University of Chittagong.

2.2. Preparation of extract

Each of the plant materials was dried and ground into powder (40–80 mesh, 700 g), and soaked for 7 days with 2–3 days interval in 3 L of methanol (*C. recurvata*, *A. bulbifer*, *T. grandiflora*) and

ethanol (*S. colocasiifolia*) at room temperature (23.0 ± 0.5) °C. Filtrate obtained through cheesecloth and Whatman filter paper No. 1 was concentrated under reduced pressure at the temperature below 50 °C using rotary evaporator (RE 200, Sterling, UK). The extracts (yield 3.8%–7.0% w/w) were all placed in air tight glass tube. About 800 mg each of the extracts was suspended in 10 mL distilled water and the suspension was shaken vigorously on a vortex mixer. These extracts were prepared to check hypoglycemic effect. The concentration (33.33 mg/mL) of extracts was prepared for screening the antibacterial properties.

2.3. Chemicals and machineries

All other chemicals and reagents were of analytical grade. Ethanol and methanol were purchased from Merck (Germany). Rapid View™ (blood glucose monitoring system, model: Bio-M1, Bioussa Inc, California, USA) with strips was purchased from Andorkilla, Chittagong. Glibenclamide was obtained from Square Pharmaceutical Ltd., Bangladesh. Nutrient agar was purchased from Merck, India. Kanamycin (30 µg/disc, Oxoid, England) was used as a standard antibiotic disc.

2.4. Animals and experimental set-up

Swiss albino mice, weighing about 28–35 g, were collected from Jahangir Nagar University, Savar, Bangladesh. The animals were furnished with standard lab nourishment and refined water *ad libitum* and maintained at natural regular day-night cycle having legitimate ventilation in the room. All the experiments were conducted in an isolated and noiseless condition. The study protocol was approved by the P&D Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh. The mice were acclimatized to laboratory condition for 7 days prior to experimentation. All animal experiments were carried out according to the guidelines of Institutional Animals Ethics Committee and study protocols were approved by the Department of Pharmacy, International Islamic University Chittagong Medical Ethics, Biosafety.

2.5. Acute toxicity study

For acute toxicity study, 40 Swiss albino female mice were used. According to the previous method, mice were divided into four groups of five animals each[14]. Different doses (1 000 mg/kg, 2 000 mg/kg, 3 000 mg/kg and 4 000 mg/kg) of different extracts of examined plants were administered by stomach tube. Then the animals were observed for general toxicity signs.

2.6. Hypoglycemic effect in normal mice

Mice were kept fasting overnight with free access to water. Group I was treated as control group, Group II was treated with glibenclamide (5 mg/kg body weight), Group III-VI were treated with methanol extract of *C. recurvata* roots (MECR), methanol extract of *A. bulbifer* leaves (MEAB), methanol extract of *T. grandiflora* whole plant (METG) and ethanol extract of *S. colocasiifolia* leaves (EESC) at 800 mg/kg body weight respectively. Before administration of drug and extracts solutions, fasting blood glucose levels were estimated by glucose oxidase method[15,16]. Then blood glucose levels were again estimated after 2 h of administration of drug and extract solutions. Glucose levels were measured by rapid View™ (blood glucose monitoring system, model: Bio-M1, Bioussa Inc, California, USA). The maximum hypoglycemic effect of glibenclamide was

found after 2 h of its administration. Percent decrease of blood glucose level after 2 h was measured by following equation:

$$\text{Decrease (\%)} = \frac{\text{GL}_{\text{before}} - \text{GL}_{\text{after}}}{\text{GL}_{\text{before}}} \times 100$$

where, $\text{GL}_{\text{before}}$ = blood glucose level before drug or extract and fractions administration and GL_{after} = blood glucose level after drug or extract and fractions administration.

2.7. In vitro antibacterial activity

2.7.1. Microorganisms

Seven bacterial species included Gram-positive [*Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Bacillus cereus* (*B. cereus*)] and Gram-negative [*Salmonella typhi* (*S. typhi*), *Salmonella paratyphi* (*S. paratyphi*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*)]. These microbes were obtained from the department of Pharmacy International Islamic University Chittagong.

2.7.2. Media preparation and maintenance of bacteria

All of the bacterial strains were grown and maintained on Nutrient agar (Merck, India) media at 37 °C and pH 7.4 ± 0.2. The bacteria were subculture overnight.

2.7.3. Preparation of concentration

In the study of the antibacterial activity, all the extracts were diluted in their solvent. So methanol extract was diluted in methanol and also other. The concentrations corresponding to the extracts were expressed in terms of µg/disk.

2.7.4. Preparation of discs

The discs of about 5 mm in diameter were cut by punching machine from Whatman No.1 filter paper. The discs were taken in a Petri dish and sterilized by autoclaving, and dried in oven at 180 °C.

2.7.5. Antimicrobial screening by disk diffusion technique

The antibacterial effects were tested by the disc diffusion method with some minor modification[17,18]. The filter paper discs (5 mm in diameter) were individually impregnated with 24 µL of 800 µg/disk and 30 µL of 1 000 µg/disk of different plant extracts and then placed onto the agar plates which had previously been inoculated with the test microorganisms (within 15 min). The Petri dishes were kept at 4 °C for 3 h before incubation at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimeters. All the tests were performed in duplicate. Blank disc impregnated with methanol, ethanol and distilled water was used as negative control and disc of kanamycin (30 µg/disc) was used as positive control.

2.7.6. Determination of relative percentage inhibition

The relative percentage inhibition of the test extract with respect to positive control was calculated by using the following formula[19,20].

$$\text{Relative percentage inhibition (\%)} = \frac{100 \times (x - y)}{z - y}$$

where, x = total area of inhibition of the test extract; y = total area of inhibition of the solvent; z = total area of inhibition of the standard drug. The total area of the inhibition was calculated by using area = πr^2 (r = radius of zone of inhibition).

2.8. Statistical analysis

The results were expressed as mean ± SD for the zone of inhibition and results were expressed as mean ± SEM from triplicate experiments for hypoglycemic effect and evaluated with the Dunnett's test. Differences were considered significant at a level of $P < 0.05$, $P < 0.01$ and $P < 0.001$.

3. Results

3.1. Acute toxicity study

None of the animals showed behavioral, neurological or physical changes characterized by symptoms such as reduced motor activity, restlessness, convulsions and coma at the limit dose of 4 000 mg/kg of different extracts during the observation period. In addition, no mortality was observed at the test dose. Thus, LD_{50} of all plant extracts was found to be greater than 4 000 mg/kg.

3.2. Hypoglycemic effect in normal mice

Among all the extracts, only EESC leaves reduced blood sugar level in normal mice. Other extracts didn't show any reduction of blood sugar level, but they increased sugar level. Glibenclamide showed significant reduction at level of $P < 0.01$. Dose of 800 mg/kg EESC showed significant reduction at level of $P < 0.01$, compared with control, Dunnett's test. These results suggested that hypoglycemic activity of EESC at 800 mg/kg dose and glibenclamide had similar significance level. All results were presented in Table 1 and percentage of decrease of blood glucose level in normal mice after 2 h with different treatment was shown in Table 1. EESC at a dose of 800 mg/kg significantly decreased blood glucose level (25.13%) than other treatments, accepting standard glibenclamide.

Table 1

Effect of MECR, MEAB, METG and EESC on fasting blood glucose level (mmol/L) in normal mice.

Groups	Dose (oral)	Before administration	After administration	Decrease (%)
Control (1% Tween)	10 mL/kg	4.090 ± 0.231	4.780 ± 0.411	-
Glibenclamide	5 mg/kg	4.640 ± 0.341 ^a	2.790 ± 0.178 ^{b,y}	39.63
MECR	800 mg/kg	6.300 ± 0.687 ^a	6.920 ± 0.806 ^a	-
MEAB	800 mg/kg	4.680 ± 0.785 ^a	5.500 ± 0.809 ^a	-
METG	800 mg/kg	5.200 ± 0.189 ^a	5.900 ± 0.297 ^a	-
EESC	800 mg/kg	5.720 ± 0.252 ^a	4.560 ± 0.178 ^{b,y}	20.28

Values were presented as mean ± SEM (n = 5). Values in same row with different superscripts were significantly different (^a $P > 0.05$, ^b $P < 0.01$). Paired *t*-test was performed to analyze before and after relationship. Values with different superscripts in same column were significantly different from control after the administration of standard and different doses of the extract and fractions (^z $P < 0.01$). One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this comparison. -: No decrease.

3.3. In vitro antibacterial activity

3.3.1. Determination of zone of inhibition

The antibacterial activity of MECR, MEAB, METG and EESC were tested against 7 pathogenic bacteria, and MECR, MEAB and EESC exhibited a significant antibacterial activity against both Gram-positive and Gram-negative bacteria at the concentration

Table 2
Results of antibacterial activity testing of MECR, MEAB, METG and EESC. mm.

Name of the bacteria	Solvents		MECR		MEAB		METG		EESC		Kanamycin
	Methanol	Ethanol	1000 µg/disc	800 µg/disc	1000 µg/disc	800 µg/disc	1000 µg/disc	800 µg/disc	1000 µg/disc	800 µg/disc	30 µg/disc
Gram-positive <i>S. aureus</i>	-	-	10.20 ± 1.04 ^a	7.30 ± 0.29 ^a	-	-	-	-	11.20 ± 1.26 ^{a*}	9.30 ± 0.29 ^a	22.20 ± 0.76
<i>B. subtilis</i>	-	-	-	-	-	-	-	-	12.00 ± 0.50 ^{a*}	9.00 ± 0.50 ^a	18.20 ± 0.29
<i>B. cereus</i>	-	-	10.50 ± 0.50 ^{b*}	8.50 ± 0.50 ^b	8.50 ± 0.50 ^b	7.50 ± 0.50 ^b	-	-	10.30 ± 0.58 ^b	8.30 ± 0.58 ^b	25.00 ± 0.50
Gram-negative <i>S. typhi</i>	-	-	16.20 ± 1.26 ^a	10.80 ± 0.29 ^b	10.00 ± 0.50 ^b	8.30 ± 0.58 ^b	-	-	12.50 ± 0.50 ^b	9.50 ± 0.50 ^b	25.30 ± 0.58
<i>S. paratyphi</i>	-	-	10.20 ± 0.58 ^a	8.50 ± 0.50 ^b	10.20 ± 0.76 ^a	7.80 ± 0.76 ^a	-	-	10.80 ± 0.29 ^{b*}	8.30 ± 0.58 ^a	20.30 ± 0.29
<i>E. coli</i>	-	-	13.00 ± 1.00 ^{a*}	11.50 ± 0.87 ^a	10.30 ± 1.04 ^a	8.50 ± 0.50 ^b	-	-	-	-	23.50 ± 0.50
<i>P. aeruginosa</i>	-	-	-	-	8.50 ± 0.50 ^{b*}	7.20 ± 0.76 ^b	-	-	-	-	25.50 ± 0.50

Values were expressed as mean ± SD of three replicates of inhibition zone (mm). ^a: The highest antibacterial activity of extracts on each test bacteria; ^{a, b}: The values were significantly different (^a*P* < 0.01 and ^b*P* < 0.001) as compared with standard (kanamycin) in same row in Dunnett's test by SPSS; -: No zone of inhibition.

of 800 and 1000 µg/disc which was shown in Table 2. The inhibitory activities shown the test samples were compared with standard broad spectrum antibiotic kanamycin (30 µg/disc). The zone of inhibition produced by MECR against Gram-positive bacteria were found to be (7.30 ± 0.29) mm to (10.50 ± 0.50) mm and against Gram-negative bacteria were found to be (8.50 ± 0.50) mm to (16.20 ± 1.26) mm at different concentrations. But *P. aeruginosa* showed resistant for MECR. MEAB produced (7.50 ± 0.50) mm and (8.50 ± 0.50) mm zone of inhibition against Gram-positive bacteria *B. cereus* at 800 and 1000 µg/disc. But MEAB didn't show zone of inhibition for *S. aureus* and *B. subtilis*. EESC produced zone of inhibition against Gram-positive bacteria in the range of (8.30 ± 0.58) mm to (12.00 ± 0.50) mm and against Gram-negative bacteria in range of (8.30 ± 0.58) mm to (12.50 ± 0.50) mm at different concentrations. *E. coli* and *P. aeruginosa* showed resistant for EESC. METG had no effect on the tested bacteria. On the other hand, kanamycin showed a zone of inhibition against Gram-positive bacteria in the range of (18.20 ± 0.29) mm to (25.00 ± 0.50) mm and against Gram-negative bacteria in the range of (20.30 ± 0.29) mm to (25.50 ± 0.50) mm.

3.3.2. Determination of relative percentage inhibition

The results of antimicrobial activity of plant extract were compared with the positive control (standard drugs) for evaluating their relative percentage inhibition. The four organic extracts exhibited maximum relative percentage inhibition against the tested bacteria were presented in Table 3.

Table 3
Relative percentage inhibition of different extracts with their doses compared to standard antibiotics. %.

Name of the bacteria	MECR		MEAB		METG		EESC	
	1000 µg/disc	800 µg/disc						
Gram-positive <i>S. aureus</i>	21.2	10.8	0.0	0.0	0.0	0.0	25.5	17.6
<i>B. subtilis</i>	0.0	0.0	0.0	0.0	0.0	0.0	43.6	24.5
<i>B. cereus</i>	17.6	11.6	11.6	9.0	0.0	0.0	16.9	11.1
Gram-negative <i>S. typhi</i>	40.9	18.2	15.6	10.7	0.0	0.0	24.4	14.1
<i>S. paratyphi</i>	25.2	17.5	25.0	14.7	0.0	0.0	28.2	16.7
<i>E. coli</i>	30.6	23.9	19.2	13.1	0.0	0.0	0.0	0.0
<i>P. aeruginosa</i>	0.0	0.0	11.1	8.0	0.0	0.0	0.0	0.0

Values were calculated from their mean values.

4. Discussion

Developing agents for treatment of diabetes mellitus that are less of adverse effects are still a challenge to the medical care system. Diabetes mellitus have long been assumed to be related to chronically elevated blood glucose levels. And it causes

disturbances in the uptake of glucose as well as glucose metabolism. Consequently, research is progressively done on medicinal plants with the hope of developing a relatively safe antidiabetic plant-based product alone or in combination with other agents[21].

In this study ethanol extract of leaves of *S. colocasiifolia* exerted significant hypoglycemic activity in fasting glucose level reduction in normal mice. Decrease of blood glucose level after 2 h of treatment is very well significant compared with control. To our best knowledge, this is the first study about hypoglycemic activity of *S. colocasiifolia*. That's why the exact mode of action is not determined yet.

Other plant extract of *C. recurvata*, *A. bulbifer* and *T. grandiflora* showed no hypoglycemic effect, though they exhibited hyperglycemic effect. After the administration of these extracts in normal mice, blood glucose level after 2 h increased. It happened maybe because of their phytoconstituent. Some phytoconstituents have acted as glucose and if a extract contains this type of phytoconstituents, they obviously increase glucose level in the blood.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay[22,23]. That's why our goal was to identify the antibacterial activity of *C. recurvata*, *A. bulbifer*, *T. grandiflora* and *S. colocasiifolia*. From the results, it is clear that *T. grandiflora* has no antibacterial effect. Because its extract didn't show any zone of inhibition against tested bacterial. But *C. recurvata* and *S. colocasiifolia* have moderate antibacterial effect. MECR and EESC both showed maximum zone of inhibition against three pathogenic bacteria at their 1000 µg/disc dose. MECR showed maximum zone of inhibition against *B. cereus* [(10.50 ± 0.50) mm], *S. typhi* [(16.20 ± 1.26) mm] and *E. coli* [(13.00 ± 1.00) mm], and EESC showed maximum zone of inhibition against *S. aureus* [(11.20 ± 1.26) mm], *B. subtilis* [(12.00 ± 0.50) mm] and *S. paratyphi* [(10.80 ± 0.29) mm]. They also showed dose dependant antibacterial activity. Only MEAB showed (8.50 ± 0.50) mm and (7.20 ± 0.76) mm zone of inhibition against *P. aeruginosa* at 1000 and 800 µg/disc dose respectively. Relative percentage inhibition of zone of inhibition is moderate to high (7.97%–43.6%) compared with standard kanamycin (30 µg/disc).

These outcomes suggested that only EESC possesses a hypoglycemic principle and can be useful for the treatment of diabetes. And for the development of new antimicrobial agent, *C. recurvata*, *A. bulbifer* and *S. colocasiifolia* is potential competitor. Further studies are warranted to isolate the active principle and to find out its accurate mechanism of action.

The results obtained in the present study demonstrated that a

number of Bangladeshi vegetables have promising antibacterial activity against tested microorganisms which can be used in the healing of various infectious diseases caused by resistant microorganisms. Although *C. recurvata* and *S. colocasiifolia* showed comparable high antibacterial activity and *A. bulbifer* showed moderate activity, further studies should be needed to develop new antibacterial agent from them. But we can exclude *T. grandiflora* for further investigation for hypoglycemic and antibacterial activities, though it had not showed that kind of activities. And it was found that *S. colocasiifolia* could be considered as very promising and beneficial hypoglycemic agent. *S. colocasiifolia* may be a potential source for the development of new oral hypoglycemic agent.

Conflict of interest statement

We declare that we have no conflict of interest.

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