Assessment of the laxative activity of an ethanolic extract of *Bambusa arundinacea* (Retz.) Willd. shoot

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Abstract**Ethnobotanical relevance**

Bambusa arundinacea (Retz.) Willd., commonly known as Kanta Bans, plays an important ethnobotanical role, especially in Asia. In traditional medicine it has reportedly been used for the treatment of constipation, blood-diseases, leucodema, inflammation and urinary discharges. A number of ethnomedicinal records exist regarding the use of *B. arundinaceae* shoots as a laxative, however, there are no scientific studies reported on its laxative activity. Therefore, the aim of this present study was to evaluate the laxative activity of an ethanolic *B. arundinaceae* shoot extract in mice.

Materials and methods

B. arundinacea shoots were collected from Dhaka, Bangladesh in July 2015. An ethanolic shoot extract was obtained and its laxative activity was evaluated by faecal consistency, gastrointestinal transit and entero-pooling assays in a mouse model. Furthermore, a phytochemical investigation of the extract was conducted by UHPLC-ESI-QqQ MS and UHPLC-ESI-Orbitrap MS analysis.

Results

The ethanolic shoot extract of *B. arundinacea* showed significant laxative activity in our mouse model, with significant increases in (i) the amount of wet faeces, with the maximum effect at 2 h for 500 mg/kg (47.92 %), (ii) gastrointestinal transit (67.18 and 60.03 % for doses of 250 and 500 mg/kg, respectively), and (iii) small intestine content at the test doses of 250 and 500 mg/kg p.o. Phytochemical investigation identified a total of thirty compounds in the ethanolic shoot extract of *B. arundinacea* using UHPLC-ESI-QqQ MS and UHPLC-ESI-Orbitrap MS analysis.

Conclusions

The results of this study provide support for the traditional use of *B. arundinacea* shoot as a laxative.

Graphical Abstract:

Keywords: *Bambusa arundinacea*; laxative; faecal consistency; gastrointestinal transit; entero-pooling assay.

1. Introduction

Constipation is a functional bowel disorder that causes discomfort in daily life, affecting about 14% of the adult population worldwide (Cirillo and Capasso, 2015; Longstreth et al., 2006; Saito et al., 2002). The use of botanicals such as senna, cascara, frangula, aloe, and rhubarb have long been utilised in the management of constipation (Cirillo and Capasso, 2015).

Bambusa arundinacea (Retz.) willd., belonging to the family Poaceae, is a thorny plant native to Asia, where it predominantly occurs throughout India, Bangladesh, China, Sri

Lanka, Myanmar and Malaya (Ghani, 2003; Ohnberger, 1999). Since the era between 12000-2000 BC, the plant has played an important role in human civilization as a structural tool and in the folk medicine in numerous tribes and locations (Inc., 2014).

The stems and leaves of the plant have been used in folk medicines to treat different inflammatory conditions (Yusuf et al., 2009). In Ayurveda the leaves, stems and roots are used as an astringent, diuretic and laxative (Nadkarni, 2000). The juice of *B. arundinacea* leaf has been used as a drink to strengthen the cartilage in sufferers of osteoarthritis and osteoporosis. It reportedly increases the integrity of the bones, arterial walls, skin, teeth, the gum, hair and nails, along with alleviating eczema and psoriasis (Vanithakumari et al., 1989). In the treatment of dysmenorrhoea and amenorrhea a decoction of leaves of *B. arundinacea* has been used to stimulate menstruation and reportedly reduces menstrual pain (Kirtikar and Basu, 1999). The shoots and spouts of *B. arundinacea* have been used in traditional medicinal systems of Bangladesh for the treatment of constipation and urinary discharges (Ghani, 2003; Yusuf et al., 2009). The plant is reported to possess antibacterial, antifungal, anti-hyperglycemic, antifertility, anti-inflammatory and antiulcer activities (Kumar et al., 2012; Muniappan and Sundararaj, 2003; Thamizharasan et al., 2015; Vanithakumari et al., 1989; Zubair et al., 2013). Phytochemical studies have indicated that the plant contains 3',3,6,7-tetramethoxy-4',5,8-trihydroxy flavones; 4-methoxy benzoic acid; 4'-hydroxy flavan-3-ol and hemicelluloses (Guha and Pant, 1967; Kumar et al., 2016). However, there are no reports to-date regarding laxative activity in an animal model. Therefore, this study was carried out to evaluate the laxative activity of the ethanolic extract of *B. arundinacea* shoot in mice.

2. Materials and methods

2.1. Plant material and extraction

Shoots of *Bambusa arundinacea* (Retz.) Willd. were collected July 2015 from Dhaka, Bangladesh and identified by taxonomists at the Bangladesh National Herbarium, where a voucher specimen (DACB 43377) has been deposited for future reference. The air-dried shoots were powdered and extracted by maceration with ethanol at room temperature for 72 h. The crude extract was obtained after filtration and evaporation of the solvent using a rotary evaporator, followed by freeze drying (yield 1.83% w/w).

2.2. Animals

Adult Swiss-albino mice of 4-5 weeks age (20-25 g) were obtained from the Animal Resources Branch of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The mice were kept in polypropylene cages under pathogen free conditions at an ambient temperature of $24\pm1^{\circ}\text{C}$; 12 h light/dark cycle and $55\pm5\%$ controlled relative humidity. The animals were fed with a standard pellet diet and water *ad libitum*. The experimental protocol followed the guidelines and regulations of the Animal Ethics Committee, Khulna University, Bangladesh (Reference no.: KU/PHARM/AEC/15/006/017).

2.3. Acute toxicity test

An acute toxicity study of the ethanolic shoot extract of *B. arundinacea* was conducted following a standard protocol (Lorke, 1983) as described in the supplementary materials.

2.4. Grouping and extract dosing

Animals of either sex were randomly divided into 4 groups ($n = 5$ mice). The negative control group received vehicle (1% Tween 80 in water) orally at a volume of 10 mL/kg b.w., while the positive control group received castor oil (0.3 mL/animal, p.o.). The other two groups (test groups, 2×5 mice) were treated orally with the *B. arundinacea* extracts (dissolved in 1% Tween 80 in water), one at a dose of 250 mg/kg b.w. and the other at a dose of 500 mg/kg

b.w., respectively.

2.5. Effect of B. arundinacea shoot ethanol extract on faecal consistency

Faecal consistency activity of the ethanol shoot extract was measured utilising a standard method (Méité et al., 2010), with some modification, as described in the supplementary materials.

2.6. Effect of B. arundinacea ethanol shoot extract on intestinal transit

Effect of *B. arundinacea* shoot extract on intestinal transit was measured as per an established method (Méité et al., 2010; Vogel et al., 2002), with minor modification, as described in the supplementary materials.

2.7. Effect of B. arundinacea ethanol shoot extract on entero-pooling

Entero-pooling activity of the *B. arundinacea* shoot ethanol extract was determined according to a standard protocol (Robert et al., 1976; Samuel et al., 2015), with minor modification, as described in the supplementary materials.

2.8. Phytochemical investigation of B. arundinacea shoot extract

The extract was subjected to phytochemical investigation using ultrahigh-pressure liquid chromatography electrospray ionisation triple-quadrupole mass spectrometry (UHPLC-ESI-QqQ MS) and ultrahigh-pressure liquid chromatography electrospray ionisation Orbitrap mass spectrometry (UHPLC-ESI-Orbitrap MS) analysis as described in the supplementary materials.

2.9. Statistical analysis

All data exhibited normal distribution and the results were expressed as mean $\pm$ SEM. We performed unpaired student's *t*-test for statistical analysis using GraphPad Prism 5.0, USA software and results were considered significant when $p < 0.05$ compared to the control. We also then performed one-way ANOVA for analysis of variance of each experiment (reported in the Supplementary Material as Table S1) to identify where these effects lie. ANOVA analysis was performed using InVivoStat 3.6, UK software.

3. Results

3.1. Acute toxicity study of *B. arundinacea* ethanol shoot extract

No mortality or symptoms of toxicity were observed in mice for the *B. arundinacea* shoot ethanol extracts under investigation even at the highest dose (0.5 g/kg) tested.

3.2. Effect of *B. arundinacea* shoot ethanol extract on faecal consistency

The results of the faecal consistency assay are reported in Figure 1. In this study, the *B. arundinacea* ethanol shoot extract showed a dose dependant increase in % wet faeces of mice (variation in the degrees of 'wet faeces' were seen) with results being statistically significant as compared to the negative control (see Figure 1). However, the effect of the shoot extract was not as strong as the castor oil (positive control) (% wet faeces was $> 50\%$ and $50 - 20\%$ up to 4 h, for castor oil and test extracts, respectively).

3.3. Effect of *B. arundinacea* ethanol shoot extract on intestinal transit

Apart from the increase in watery faeces, the ethanol shoot extract of *B. arundinacea* significantly increased the propulsion of BaSO₄ through the gastrointestinal tract in a dose-dependent manner (intestinal transit 67.2% and 60.0% at 500 and 250 mg/kg dosage, respectively) (Table 1). The effect of *B. arundinacea* shoot ethanol extract on the intestinal transit at the dose of 500 mg/kg (67.2%) and of 250 mg/kg (60.0%) was comparable to the

negative control (54.1%), while standard castor oil produced the highest gastrointestinal motility (intestinal transit 73.1%). The effects of shoot extract at both doses were statistically significant in comparison to the negative control.

3.4. Effect of *B. arundinacea* ethanol shoot extract on entero-pooling

In addition to a positive intestinal transit effect, the shoot extract was further evaluated for its effect on intestinal content. The results of the content of intestinal fluid control following administration of the ethanol shoot extract at a dose of 500, 250 mg/kg or castor oil (0.3 mL/animal, p.o.) are shown in Table 1. At both extract dosages a significant increase in small intestinal content was seen relative to the negative control (0.66 g, 0.43 g and 0.29 g for 500, 250 mg/kg and 1% Tween 80, respectively) in the test mice. The results were statistically significant in relation to the negative control. Nevertheless, the effect of the extracts was not as strong as that seen in the castor oil treated group (0.80 g, positive control group) (Table 1). The results of this experiment support our previous data obtained in the investigation of faecal consistency, since the intestinal content of the test groups were more fluidic in nature when compared to the control.

3.5 Phytochemical investigation of *B. arundinacea* shoot extract

Thirty compounds have been identified in the ethanolic shoot extract of *B. arundinacea* by analyzing their fragmentation pattern from both UHPLC-ESI-QqQ MS and UHPLC-ESI-Orbitrap MS analysis. Some of these compounds absorbed in the UV-visible region. The complete list of the identified compounds is included in the supplementary materials (Table S2 and S3) along with all chromatograms (Figures S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13 and S14).

4. Discussion

Laxatives are agents that are used in the management of constipation and usually act by enhancing retention of intraluminal fluid, decreasing net absorption of fluid, or altering motility by either inhibiting non-propulsive contractions or stimulating propulsive contractions (Brunton et al., 2007). In this study, to evaluate the medicinal use of *B. arundinacea* shoot in the treatment of constipation, the ethanolic extract of *B. arundinacea* shoot was tested in mice. The extract demonstrated significant laxative activity by promoting watery faeces, increasing gastrointestinal motility and the amount of intestinal content, when compared with a negative control and was similar in activity to our positive control, castor oil (see Figures 1 and Table 1).

Castor oil induces laxation through ricinoleic acid, a product from enzymatic hydrolysis of its triglyceride present in the oil (Watson and Gordon, 1962). Ricinoleic acid produces a hyper-secretory response resulting in water accumulation within the bowel, thereby speeding up the intestinal transit time (Hardman and Limbird, 2001). In this study, the *B. arundinacea* ethanol shoot extract administered at 500 mg/kg produced various degrees of wet faeces up to a 4 h observation period in a similar pattern to castor oil (positive control). The % wet faeces of mice increased at a dose of 250 mg/kg being observed over 2 h (41.67 %) with results being statistically significant as compared to the negative control. Of note is that the underlying mechanism/s of laxative activity of *B. arundinacea* ethanol shoot extract cannot be discerned from the above experiments. However, the detected increased wet faeces in mice of the test extracts in the faecal consistency assay can be attributed to the presence of constituents in the extract which likely acts via one of the above mechanisms, resulting in cumulative watery faeces in the test mice.

The shoot ethanol extract (250 and 500 mg/kg dosage) of *B. arundinacea* significantly increased the propulsion of BaSO₄ through the gastrointestinal tract in a dose-dependent

manner and was comparable to the negative control (Table 1). A number of physiological entities, such as acetylcholine, histamine, substance P, cholecystokinins, prostaglandins and 5-hydroxytryptamine have been proposed as mediators of this action. Release of these chemical mediators within the gut is known to increase cytosolic calcium, which in turn stimulates intestinal propulsion (Rehman et al., 2012; Shareef et al., 2014). The presence of phyto-constituents such as flavonol, phenolics and alkaloids are also reported to be responsible for laxative activities found in various plants (Longanga Otshudi et al., 2000; Yang et al., 2008). It is reported that some phytochemicals such as the above constituents, including resveratrol potentially play a key role in the laxative activity by increasing intestinal motility via acetylcholine receptors (Seo et al., 2013). A recent study found that the ethanol extract of *B. arundinacea* possesses a high phenolic content (Kaur et al., 2015), while another study identified flavonol in *B. arundinacea* (Kumar et al., 2016). The mechanism behind the increase in gastrointestinal motility by the ethanol shoot extract of *B. arundinacea* is not well defined. However, it seems reasonable that the presence of phenolic constituents in the shoot extract likely contribute to the observed increased intestinal transit activity in the tested mice. In addition to that, there is a positive correlation between increase in the intestinal motility and increase in intestinal fluid. In other words watery stools result in rapid transit (Longstreth et al., 2006). The increase in water content in faeces is a likely cause of the increase in intestinal transit seen in our results.

The results of the entero-pooling assay showed that the ethanol extract of *B. arundinacea* shoot increased small intestinal content in the test mice and that the result was comparable to the 1% Tween 80 negative control (0.66 g, 0.43 g and 0.29 g for 500, 250 mg/kg and negative control, respectively). It is reported that many laxatives act on or affect the net water flux and levels of prostaglandin E (PGE) in the animal colon (Beubler and Juan, 1979). PGEs stimulate mucus and bicarbonate secretion, increase contraction of intestinal smooth muscles,

inhibit gastric acid secretion and increase mucous secretion (Al-Fartosy, 2013; Tunaru et al., 2012). Polyphenolic compounds are also reported to have an important role in the stimulation of PGE secretion in the gastrointestinal tract (Sumbul et al., 2011). The observed increased intestinal content in test mice by the *B. arundinacea* shoot extract may be due to polyphenolic-mediated release of PGE, as these were detected in the extract as discussed *vide infra*. Additionally, a number of laxatives containing constituents such as ricinoleic acid and dioctyl sodium sulfosuccinate are reported to exert their effect by stimulation of cyclic AMP/GMP production. Such stimulation is a result of interaction of these constituents with adenylate cyclase located in the colonic mucosa and phosphorylation of cystic fibrosis transmembrane conductance regulators (CFTRs). This leads to stimulation of intestinal motility and an increase in the secretion of fluids, mainly water, and electrolytes (Cl^- and Na^+) (Capasso et al., 1988; Ratnaik et al., 2000; Simon and Kather, 1980). The observed increase in intestinal content following administration of the extract on the entero-pooling assay may therefore be mediated through stimulation of cyclic AMP/GMP production and phosphorylation of CFTRs.

Phytochemical profiling of *B. arundinacea* shoot extract using UHPLC-ESI-QqQ MS and UHPLC-ESI-Orbitrap MS analysis identified a total of thirty previously known compounds belonging to fatty acids (hydroxylated and non-hydroxylated), glycosides, anthocyanins, phytosterols, polyphenols and ginsenosides (see Tables S2 and S3). However, none of the identified specific compounds have been reported before as possessing laxative activity. In general, except fatty acids none of the identified chemical class of compounds also been reported to possess laxative activity. The ability of fatty acids to induce laxation is long established and a number of reports exist showing a positive correlation between the amounts of fatty acid in the diet and faecal consistency (Donowitz et al., 1995). Both hydroxylated and non-hydroxylated fatty acids are known to diminish fluid absorption in both the human and

rodent bowel and thereby produce a net fluid and electrolyte accumulation (Binder, 1977). Fatty acids also reportedly induce a contractile effect on the animal colon (Yajima, 1985). Therefore, the presence of different fatty acids in the *B. arundinacea* ethanolic shoot extract is likely to be responsible or contributing to its laxative activity. Interestingly, hemicellulose has also been previously identified in *B. arundinacea* and is well known as a dietary fiber component with laxative effects (Dhingra et al., 2012; Yang et al., 2012). It seems reasonable then that different fatty acids, flavonols and hemicellulose at least in part are responsible for the laxative activity of *B. arundinacea* that we have observed in this study.

5. Conclusion

The results of this study showed that *B. arundinacea* shoot has laxative effects in mice and therefore provides solid evidence for its use as a laxative in traditional ethnobotanical medicine. Specifically, an increase in wet faeces, entero-pooling and an increase in small intestinal transit were observed following administration of the ethanol shoot extract in mice. However, further bioactivity guided purification of active constituents needs to be carried out to specifically identify the bioactive constituents responsible for this observed activity and their mechanism of action.

Authors' contributions

SMNKZ and MSR carried out *in-vivo* tests under the supervision of SJU and JAS. JAS and SJU designed the work and SMNKZ and HB prepared the manuscript. SS did the phytochemical profiling with UHPLC-MS and wrote the part related to that. DG helped in data analysis and revision of the manuscript.

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Ethical statement

The experimental protocol adhered to in this work followed the guidelines and regulations of the Animal Ethics Committee, Khulna University, Bangladesh. The guidelines were in accordance with the internationally accepted principle for laboratory use and care (National Research Council of USA, 1996).

Conflict of interest

The authors declare no conflict of interests.

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Figure 1. Effect of *B. arundinacea* shoot ethanol extract on faecal consistency in mice.

Results are expressed as mean $\pm$ SEM. * p <0.05 vs. control, ** p <0.001 vs. control.

Table 1. Effect of *B. arundinacea* shoot ethanol extract on intestinal transit and entero-pooling (small intestine weight) in mice.

Treatment	Dose	% Intestinal transit	Weight of small intestinal content (g)
Control (1% Tween 80 in water)	10 mL/kg	54.13 ± 1.95	0.29 ± 0.01
Positive control (Castor oil)	0.3 mL/animal	73.12 ± 1.78**	0.80 ± 0.11**
<i>B. arundinacea</i> shoot extract	500 mg/kg	67.18 ± 1.86**	0.66 ± 0.04**
	250 mg/kg	60.03 ± 0.65*	0.43 ± 0.03**

Results were expressed as mean ± SEM. * $p < 0.05$ vs. control, ** $p < 0.001$ vs. control.