

## Chemical and Biological Investigations of *Axonopus compressus* (Sw.) P. Beauv.

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The crude *n*-hexane (HX), chloroform (CF) and methanol (ME) extracts of *Axonopus compressus* (Family: Poaceae) were subjected to screenings for antioxidant, antibacterial & antifungal activities and cytotoxic against brine shrimp nauplii. The crude extracts (HX, CF and ME) did not show any antimicrobial activities, whereas, two fractions F-1 and F-2 obtained from CF extract exhibited mild to moderate sensitivity against all the test microorganisms. On the other hand, in brine shrimp lethality bioassay the LC<sub>50</sub> values of 0.323, 1.88, 2.11 and 2.50 µg/ml for vincristine sulphate (Std.), HX, CF and ME extracts, respectively indicate the presence of potent bioactive compounds. At the same time, the antioxidant potential was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent using *tert*-butyl-1-hydroxytoluene (TBHT) and ascorbic acid as reference standards. Among the crude extracts, only CF extract displayed significant antioxidant activity with IC<sub>50</sub> value of 48.0µg/ml. A compound was purified from the chloroform extract of *A. compressus* and it was identified as  $\beta$ -sitosterol on the basis of <sup>1</sup>H NMR spectroscopy as well as co-TLC with authentic sample.

*A. compressus* is usually a robust plant and stoloniferous. It has stouter culms and stolons, wider leaves and longer spikelets which are more acute. *A. compressus* has been reported for various ethnomedical uses such as haemorrhoids (Soladoye *et al.*, 2010), antimalarial (Busia, 2007; Lowe, 1989) and antidiabetic (Ibeh *et al.*, 2011) activities. It is also reported for digestible protein and high starch content (Gohl, 1975) and in Brazil, it is widely considered to be a useful feed to

gain weight for zebu steers (Rocha *et al.*, 1962). A study showed that this herb can absorb trace metals and is recommended as remedies to the pollution problem of the environment (Ukpong *et al.*, 2011). Previous phytochemical studies with *A. compressus* revealed it to contain alkaloid, flavonoids, polyphenols, saponin, inulin, cellulose and tannins (Ibeh *et al.*, 2013). However, this species growing in Bangladesh has not been studied either for phytochemical or biological purposes.

The whole plant of *A. compressus* was collected from the Curzon Hall campus of Dhaka University, Bangladesh in 2008. The clean plant material was sun-dried for several days and then oven-dried for 24 hrs at 40 °C and the dried materials were ground to a coarse powder.

About 100 gm of the coarse powder of the whole plant was successively extracted in a Soxhlet apparatus with *n*-hexane (HX), chloroform (CF) and methanol (ME) (based on increasing polarity). The volume of each extractive was reduced by using a Buchii rotary evaporator at low temperature and pressure.

An aliquot (1.0 gm) of the concentrated chloroform extract was subjected to column chromatography for fractionation over silica gel (Kieselgel 60, mesh 70-230). PTLC of a column fraction with *n*-hexane - ethyl acetate (5:1) provided  $\beta$ -sitosterol (**1**) as colorless amorphous mass.

The extractives of *A. compressus* were subjected to screenings for antibacterial activity and cytotoxicity against brine shrimp nauplii following the method developed by Bauer *et al.* (1966) and Meyer *et al.* (1982), respectively. The antioxidant potential of the extracts was

also assessed by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Brand- Williams *et al.*, 1995).

The present investigation showed that fractions F-1 and F-2 obtained from the chloroform extract have mild growth inhibitory activity against almost all the bacteria and fungi with average zone of inhibition of 8-10 mm, whereas the crude HX, CF and ME extracts did not show any antimicrobial activity at the tested concentration.

It was found from the result of the brine shrimp lethality test (Table 2) that the crude extracts (HX, CF and

ME) of *A. compressus* exhibited toxicity towards brine shrimp. There was no mortality in the negative control group indicating the validity of the test. LC<sub>50</sub> obtained from the best-fit line slope were 0.3229, 1.88, 2.11 and 2.50 µg/ml for vincristine sulphate (Std.), HX, CF and ME extracts, respectively. In comparison to positive control (vincristine sulphate), the *n*-hexane, chloroform and methanol extracts were found to be highly cytotoxic. This indicates that the plant might have antitumour or pesticidal compounds.

**Table 1. Antimicrobial activity of *A. compressus*.**

Test microorganisms	Diameter of zone of inhibition (mm)		
	F-1	F-2	Kanamycin
<i>Bacillus cereus</i>	9	8	26
<i>B. megaterium</i>	10	8	24
<i>B. subtilis</i>	9	8	25
<i>Escherichia coli</i>	-	-	25
<i>Pseudomonas aeruginosa</i>	7	7	23
<i>Salmonella Paratyphi</i>	9	8	25
<i>S. Typhi</i>	8	9	25
<i>Sarcina lutea</i>	8	7	22
<i>Staphylococcus aureus</i>	-	-	23
<i>Shigella boydii</i>	7	7	23
<i>Sh. dysenteriae</i>	7	7	25
<i>Vibrio mimicus</i>	8	8	24
<i>V. parahaemolyticus</i>	8	7	25
<i>Aspergillus niger</i>	8	8	25
<i>Candida albicans</i>	8	7	25
<i>Saccharomyces cerevisiae</i>	7	7	23

A diameter less than 8 mm was considered as inactive.

**Table 2. Brine shrimp lethality of the crude extracts of *A. compressus*.**

Sample	LC <sub>50</sub> (µg/ml)	Regression equation	R <sup>2</sup>
VS	0.32	y = 29.797x + 64.624	0.927
HX	1.88	y = 23.233x + 43.619	0.915
CF	2.11	y = 25.961x + 41.537	0.961
ME	2.50	y = 24.748x + 40.140	0.983

VS: vincristine sulphate (Std.).

The crude extracts (HX, CF and ME) of *A. compressus* were also subjected to free radical scavenging activity to evaluate the antioxidant potential by using *tert*-butyl-1-hydroxytoluene (TBHT) and ascorbic acid as reference standards. The results are summarized in Table-3. It has been observed that the CF extract showed the highest

antioxidant activity with IC<sub>50</sub> value of 48.0µg/ml and it is clearly evident that the CF extract might play important roles in preventing free radical induced-diseases. On the other hand, both HE and ME extracts showed mild antioxidant activity, with IC<sub>50</sub> values of 292.0 µg/ml and 188.0 µg/ml, respectively.

From the above investigations it has been found that different solvent extracts of *A. compressus* have significant cytotoxic activity and the partially purified fractions (F-1 and F-2) obtained from the chloroform extract have mild antimicrobial activity. The high lethality to brine shrimp nauplii by all extracts is a clear indication of the presence of antitumour or pesticidal compounds. In the present study, we have isolated  $\beta$ -sitosterol and detailed chemical investigation of this plant is further required to isolate the molecules that are responsible for cytotoxicity, antimicrobial and antioxidant activities.

**Table 3. IC<sub>50</sub> values of standard and test samples of *A. compressus*.**

Sample	IC <sub>50</sub> (µg/ml)
TBHT (Standard)	27.5 ± 0.54
Ascorbic acid	5.8 ± 0.21
HX	292.0 ± 2.03
CF	48.0 ± 0.96
ME	140.0 ± 1.03

The values of IC<sub>50</sub> are expressed as mean ± SD (n=3). TBHT: *tert*-butyl-1-hydroxytoluene.

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