Short communication

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Exploring the Phytochemicals, Antioxidant Capacity and Cytotoxicity of *Abroma augustum* (L.) L.f. Seed Extract, an *In vitro* Study

Homayra Binta Sujaye, Alam Khan[1], Md. Enamul Haque[2], Md. Abdul Momin[3], Biswanath Sikdar and Md. Faruk Hasan[3]*

Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh

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The antioxidant activity of *Abroma augustum* extract was tested via an *in vitro* method using zebrafish embryos in a manner similar to that used for the cytotoxicity assay. Important phytochemicals like, alkaloids, carbohydrates, flavonoids, glycosides, phenols, polyphenols and tannins were identified from methanol extract of *A. augustum* seed extract. In terms of antioxidancy, the highest 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition rates were 65.30 ± 0.9% and 80.85 ± 1.0% for *A. augustum* seed extract and tert-butyl-1-hydroxytoluene (BHT), respectively. The concentration that inhibits 50% (IC_{50}) value of the seed extract was 105.57 ± 1.19 and the BHT value was 88.89 ± 1.0 µg/ml. In the cytotoxicity test, at 96 hrs post fertilization (hpf), treatment with 100 µg/ml seed extract resulted in yolk sac edema, tail deformation and pericardial edema. The present study may be helpful to screen for major classes of phytochemicals and to evaluate the antioxidant and cytotoxic activities of *Abroma augustum* seed extract. The present findings may also be helpful for the development of anticancer and antimicrobial drugs design in the near future.

Abroma augustum (L.) L.f. (Malvaceae) is an evergreen potential medicinal shrub that is cultivated in the Asian subcontinent, India and Bangladesh (Miah et al. 2020, Jena et al. 2023). Since the beginning of human civilization, plants have been used for the treatment of diseases as substitutes for chemically synthetic drugs. At present, human life is becoming a crucial cause of mortality and morbidity worldwide (Rahmatullah et al. 2010).

*Author for correspondence: <faruk_geb@ru.ac.bd>. [1]Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh. [2]Department of Biotechnology and Genetic Engineering, Gopalganj Science and Technology University, Gopalganj-8100, Bangladesh. [3]Department of Microbiology, University of Rajshahi, Rajshahi-6205, Bangladesh.

The plant contains several phytochemical compounds, such as triterpenes, steroids, benzohydrofurans, megastigotes, phenylethanoid glycosides and fucose (Jayasinghe and Jayawardena 2019, Roy et al. 2022). Diabetes, leucorrhoea, scabies, gonorrhea, cough, leukoderma, jaundice, headache, stomachache, dermatitis, hypertension and uterine disorders are treated with the metabolites obtained from this plant (Al-bari et al. 2006). Previously, abromine was identified as the functional component of *A. augustum* because of its antihyperglycemic activity in rabbits (Mir et al. 2013). *A. augustum* leaves have been reported to be potent antimicrobial, cytotoxic and anti-inflammatory agents for new drug establishment (Khanra et al. 2017, Sunitha et al. 2018). *A. augustum* leaf-derived silver nanoparticles have strong antibacterial and antibiofilm effects (Kumar et al. 2023). The bark extract of *A. augustum* has shown potential for promoting the apoptosis of Ehrlich ascites carcinoma cells (Jena et al. 2023). Taraxerol and squalene compounds isolated from *A. augustum* provided good anti-inflammatory potency in rats (Latief et al. 2020). Recently, anthrombolytic compounds were identified from *A. augustum* (Ivy et al. 2021). *A. augustum* seed extract exhibited pesticidal (Sujaye et al. 2023) and antimicrobial activities (Kulsum and Islam 2019). From the available information it is understood that the antioxidant and cytotoxic effects of the methanol extract of *A. augustum* seeds have not been well characterized. Hence, the present study was designed to detect the phytochemical compounds, the antioxidant activities and cytotoxic effects of seed extracts of *A. augustum*.

For this present study, *Abroma augustum* plant seeds were collected during late summer from Kajla, Motihar, Rajshahi-6206, and kindly identified by Dr. Md. Mahbubur Rahman, Department of Botany, University of Rajshahi, Bangladesh. A voucher specimen was deposited in the Botany Herbarium (Voucher No. AC205). Mature seeds were washed with distilled water and allowed to air dry for 5 days. After drying, the seeds were ground to a fine powder via a grinding machine (Nova, India). A total of 25 g of powder was then rinsed with 250 ml of methanol. The mixture was incubated at 37°C for 10 days with occasional shaking. The extract was passed through Whatman No. 1 filter paper and then extract was evaporated for 2 hrs at 60°C using a rotary evaporator (iGene Labserve, New Delhi, India) to afford a blackish mass. The completely evaporated extract was subsequently collected in a 2 ml eppendorf tube and stored at 4°C for investigations.

Phytochemical detection was performed on *A. augustum* seed extracts in methanol via standard processes as previously reported (Jabeen et al. 2023, Thakur and Kumari 2022, Yunitasari et al. 2022). To detect different phytochemicals, 5 g of crude extract was added separately for each test. The detected phytochemicals in *A. augustum* seed extract were measured via previous techniques, as described by Hazra et al. (2021) and Longbap et al. (2018). To quantify the detected phytochemicals, 10 g of extract was added individually for each test.

The free radical scavenging ability of *A. augustum* seed extract was evaluated via a previously described method (Scorsatto et al. 2019). Briefly, 3 ml of the reaction mixture

was prepared with 1 ml of 0.3 mM DPPH solution, 1 ml of seed extract and 1 ml of ethanol. The mixture was subsequently incubated at room temperature for 30 min in the dark. The absorbance was measured at 517 nm using BHT as a standard. The percentages (%) of inhibition were measured via the following equation (Scorsatto et al. 2019):

$$\text{Percentages (\%)} = (A_0\text{-}A_1/A_0) \times 100$$

Here, A_0 represents the absorbance of the control sample, and A_1 represents the absorbance of the sample.

The cytotoxicity of *A. augustum* seed extract on zebrafish embryos was studied via the method of Souza et al. (2023). Initially, different concentrations of extracts (50, 100, 150 and 200 µg/ml) were applied for standardization of effectiveness. From the initial test record, 100 µg/ml seed extract was selected as the final concentration. Three replicate treatment groups (3 × 30 embryos) were assayed in 96-well microplates and incubated at 27°C ± 1.0°C. DMSO (1%) was used as a control. The embryos were observed and pictures were taken at 25, 50 and 96 hpf via a light microscope (Olympus SZX10, Japan) at 100X magnification. The mean values were calculated from three different experiments, and the data are presented as the standard error of the mean (mean ± SEM) with SPSS software version 17 (SPSS, Chicago, IL, USA).

In the phytochemical screening, alkaloids, carbohydrates, flavonoids, glycosides, phenols, polyphenols and tannins were detected in the *A. augustum* seed extract. Additionally, the amino acids anthraquinone, phytosterols, reducing sugars, saponins, steroids and terpenoids were not detected in the seed extracts (Table 1). In the quantitative analysis of phytochemicals, the highest concentration of total phenols was 9.11%, followed by 8.76% for polyphenols in 1 g of seed extract. The lowest concentration of total carbohydrates was 2.43% with the same amount of extract (Fig. 1). Previously, similar phytochemicals were detected by Hazra et al. (2021) in the stem bark of *A. agusta* plants. These findings support our present results. In contrast, the amount of total phytocompounds may differ somewhat from the present findings due to the quantity of secondary metabolites, topographical disparity, period of the investigations, or extraction technique (Das et al. 2012). Khatoon et al. (2022) reported different concentrations of total phenols, flavonoids, saponins and alkaloids in *Tabernae montana* root extracts. Similar findings were observed in Retama plant extract by Alfalluos et al. (2017). In contrast, low concentrations of total phenolics (2.21 ± 0.39%), flavonoids (0.51 ± 0.02%), tannins (0.88 ± 0.02%) and alkaloids (1.38 ± 0.37%) were observed in *A. augustum* stem bark extracts (Hazra et al. 2021).

The methanol extract of *A. augustum* seeds showed the highest scavenging of 65.30 ± 0.9% DPPH, whereas the standard BHT value was 80.85 ± 1.0% at a concentration of 200 µg/ml. In addition, the percentages of DPPH inhibition by the seed extract and BHT were 20.95 ± 1.5% and 46.98 ± 1.5%, respectively, at a concentration of 50 µg/ml.

Table 1. Phytochemical screening of methanol extracts of *Abroma augustum* seeds.

Phytochemicals Test	Reagents	Indicators*
Alkaloids	Mayer's test	+
Amino acid	Ninhydrin test	-
Anthraquinone	Ammonium hydroxide test	-
Carbohydrate	Benedict's test)	+
Flavonoids	Alkaline reagent test	+
Glycosides	Keller–Kiliani test	+
Phenols	Potassium dischomate	+
Phytosterols	Salkowski Test	-
Polyphenol/Tannins	Ferric chloride test	+
Reducing sugar	Fehling test	-
Saponins	Foam test	+
Steroids	Liebermann-Burchard test	-
Triterpenoids	Salkowski's test	-

*, (+) = present, (-) = not present.

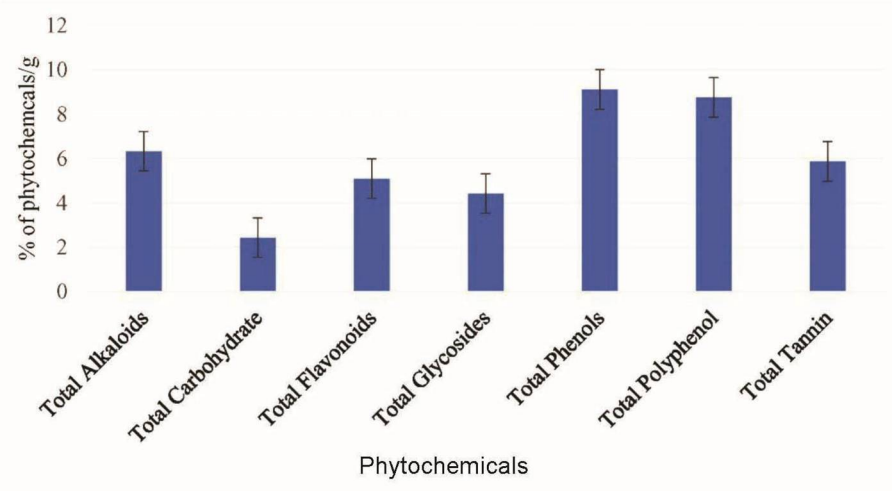

Fig. 1. Total alkaloid, carbohydrate, flavonoid, glycoside, phenol, polyphenol and tannin contents in the extract of 1 g of seed.

The IC_{50} values of the seed extract and BHT were 105.57 ± 1.19 and 88.89 ± 1.0 µg/ml, respectively (Fig. 2). Closely related results were reported in leaf extracts of *A. augustum* and *Andrographis paniculata* (Alfalluos et al. 2017, Yadav et al. 2022). In contrast, Sunitha et al. (2018) reported an IC_{50} value of 790 ± 3.6 µg/ml for the Malaysian *A. augustum* plant extract, while, Ivy et al. (2021) found IC_{50} value using 36.70 ± 0.32 µg/ml crude extract of *A. augustum* leaves. These data support our present findings.

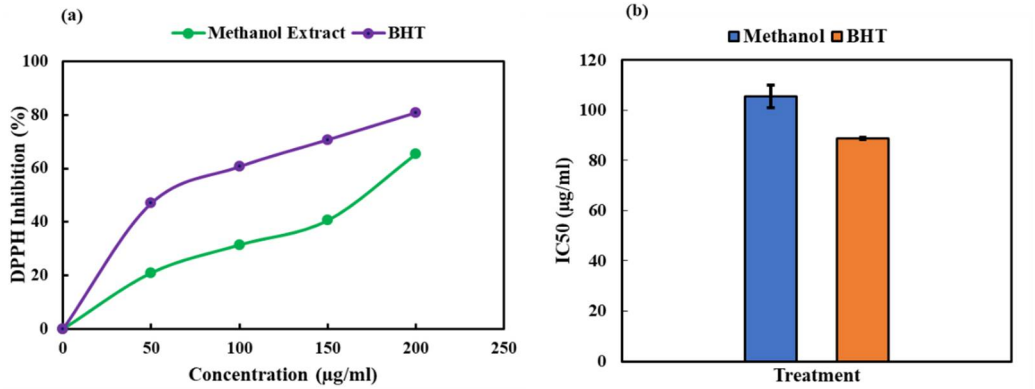

Fig. 2. Antioxidant effects of *A. augustum* seeds, where: (a) DPPH scavenging percentage and (b) IC_{50} (concentration that inhibits 50%) value.

At 25 hpf, the application of 100 µg/ml seed extract to the zebrafish embryos resulted in abnormal development during the cleavage, blastomaring and gastrulation periods. At 50 hpf, the normally hatched embryo was subjected to its normal length and the eyes and skin pigments were clearly visible in the control. Seed extracts cause deformities in zebrafish embryos, and the eyes are not clearly visible. At 96 hpf, normally grown embryos present normal skin pigments, shapes and sizes, clearly visible eyes, normal yolk sacs and elongated spines. Seed extract treatment effectively caused yolk sac edema, pericardial edema and tail deformities in zebrafish embryos, whereas the control group did not present any abnormalities (Fig. 3). Makkar et al. (2018) reported the toxicity of dental bioceramics in embryonic zebrafish. *Ficus glomerata* aqueous extract treatment showed slightly detached tails of zebrafish embryos after 24 hpf (Ismail et al. 2017). Mecanine and sophocarpine resulted in pericardial edema, tail malformation, notochord malformation, scoliosis, yolk edema, and growth retardation in zebrafish embryos after 48 hrs of treatment (Shaikh et al. 2019). *Curcuma longa* extract has teratogenic effects, body deformities, and enlarged yolk sacs at 96 hpf in zebrafish embryos (Lu et al. 2014). Safflower caused pericardial and yolk-sac edema at 96 hpf in zebrafish embryos at a concentration of 345.6 mg/l (Alafiatayo et al. 2019). Similar effect was found at 96 hpf using 1.6-2.4 mg/ml of penconazole (Xia et al. 2017).

In contrast, natural cosmetic ingredient mixtures have shown no cytotoxicity or toxicity to zebrafish embryos at high concentrations (Aksakal and Abdulkadir 2018). Wibowo et al. (2018) reported no toxicity to zebrafish embryos treated with pomegranate peel extract (196.037 ± 9.2 µg/ml) at 96 hpf. Moreover, some chemical substances have different effects on the developmental stages of zebrafish embryos (Ismail et al. 2017). Phenolic compounds universally exist in plants and are considered to have high antioxidant ability and free radical scavenging capacity (Ha et al. 2021). Polyphenol-rich plant extracts present morphological malformations, *Viz.* Spinal curvature, pericardial edema, and developmental delay may occur (Bernardo and Connaughton 2022).

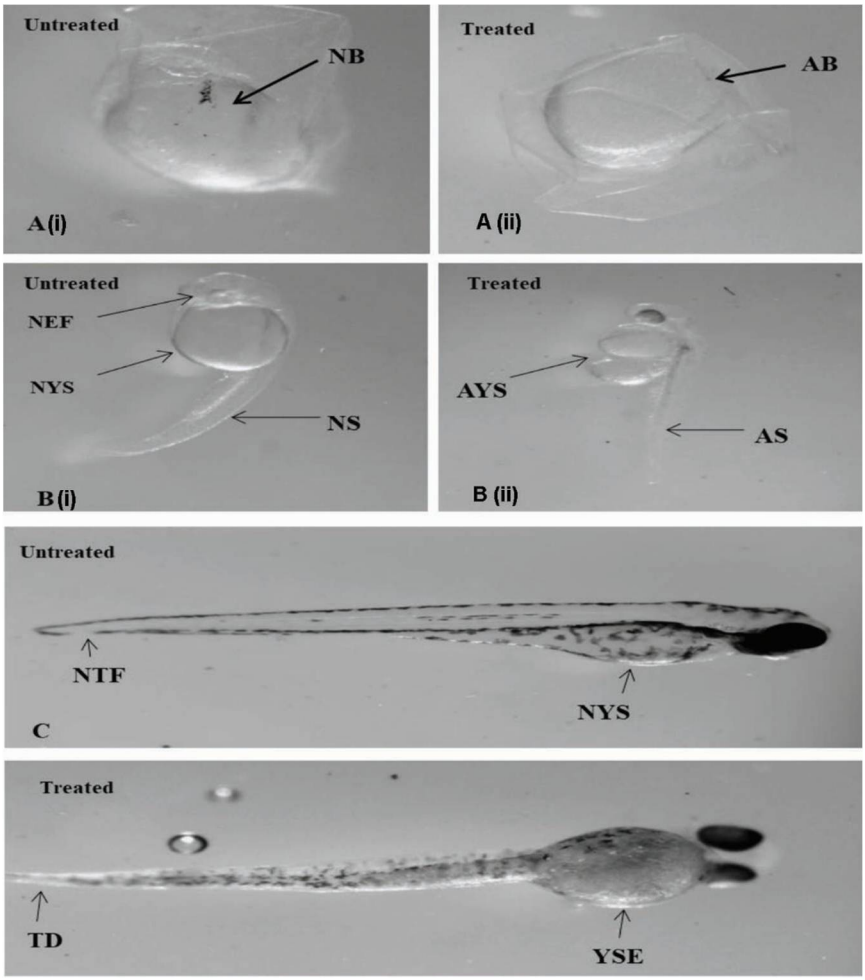

Fig. 3. Zebrafish embryo development in the untreated and seed extract-treated groups: (Ai-Aii) 25 hpf, (Bi-Bii) 50 hpf and (C) 96 hpf. Here, a normal blastomere (NB), abnormal blastomere (AB), normal eye formation (NEF), a normal yolk sac (NYS), a normal spine (NS), abnormal eye formation (AEF), an abnormal yolk sac (AYS), an abnormal spine (AS), normal tail formation (NTF), a normal yolk sac (NYS), tail deformation (TD) and represents yolk sac edema (YSE). 100X magnification was to take the images.

Additionally, polyphenol compounds have been reported to improve hyperuricemia in zebrafish embryos (Veeren et al. 2020, Wang et al. 2024). *Abroma augustum* seeds appear to be a promising source of biologically potential composites that might be able to control human disease difficulties. Moreover, the seeds of this plant could be subjected to antioxidant activity. The methanol extract of *A. augustum* seeds contains some promising phytocompounds with significant antioxidant potency and cytotoxic effects on zebrafish embryos. Further investigations are needed to confirm the cytotoxicity that may be helpful for novel drug designs in the pharmacological industry.

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