

Exploring the phytochemicals, antioxidancy and cytotoxicity of *Abroma augustum* (L.) seed extract

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Abstract

Abroma augustum is an important medicinal plant that is conventionally used in the pharmaceutical industry to treat different diseases. The key objectives of this research were to assess the phytochemical, antioxidant and cytotoxic activities of *Abroma augustum* seeds. Phytochemical screening and quantification were performed via previous methods. The antioxidant action of *A. augustum* extract was tested via an *in vitro* method. The zebrafish embryo results were analyzed in a manner similar to that used for the cytotoxicity assay. Phytochemical profiling revealed that the methanol extract of *A. augustum* seeds contained alkaloids, carbohydrates, flavonoids, glycosides, phenols, polyphenols and tannins. In terms of antioxidant activity, the highest 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition rates were $65.30 \pm 0.9\%$ and $80.85 \pm 1.0\%$ for *A. augustum* seed extract and tert-butyl-1-hydroxytoluene (BHT), respectively. The IC_{50} value of the seed extract was 105.57 ± 1.19 , and the BHT value was 88.89 ± 1.0 $\mu\text{g/ml}$. In the cytotoxicity test, at 96 hpf, treatment with 100 $\mu\text{g/ml}$ seed extract resulted in yolk sac edema, tail deformation and pericardial edema. *A. augustum* seeds contain high concentrations of phytochemicals and have high antioxidant and cytotoxic properties. The present findings may be helpful for molecular drug design in the near future.

1 Introduction

Abroma augustum (L.) L.f. (family of Malvaceae) is an evergreen potential medicinal shrub that is cultivated in the Asian subcontinent, India and Bangladesh [1, 2]. 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 [3]. The plant contains several phytochemical compounds, such as triterpenes, steroids, benzohydrofurans, megastigolones, phenylethanoid glycosides and fucose [4, 5]. Diabetes, leucorrhoea, scabies, gonorrhea, cough, leukoderma, jaundice, headache, stomachache, dermatitis, hypertension, and uterine disorders are treated with this plant [6]. Previously, abromine was identified as the functional component of *A. augusta* because of its antihyperglycemic activity in rabbits [7]. *Abroma augusta* leaves have been reported to be potent antimicrobial, cytotoxic and anti-inflammatory agents for new drug establishment [8, 9]. *A. augusta* leaf-derived silver nanoparticles have strong antibacterial and antibiofilm effects [10]. The bark extract of *A. augusta* has shown potential for promoting the apoptosis of Ehrlich ascites carcinoma cells [2]. Taraxerol and squalene compounds isolated from *A. augusta* provided good anti-inflammatory potency in rats [11]. Recently, antithrombotic compounds were identified from *A. augusta* [12]. *A. augusta* seed extract exhibited pesticidal [13] and antimicrobial activities [14]. To the best of our knowledge, the antioxidant and cytotoxic effects of the methanol extract of *A. augusta* seeds have not been well characterized. Hence, we designed this study to detect the phytochemical compounds and the antioxidant and cytotoxic effects of *A. augustum* seeds.

2 Materials and methods

2.1 Chemicals and reagents used

The methanol used for plant material extraction was of high-performance liquid chromatography (HPLC) grade. Methanol, ascorbic acid, DPPH and BHT were purchased from Merck, Darmstadt, Germany. Dimethyl sulfoxide (DMSO) was purchased from Sigma–Aldrich, USA. All the reagents and chemicals used were of analytical grade.

2.2 Plant seed collection

Abroma augustum plant seeds were collected during late summer (July 2022) 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 used as plant material.

2.3 Plant material extraction

The seeds were washed with distilled water and allowed to air dry for 5 days. After drying, the seeds were ground to a fine powder using a grinding machine (Nova, India). Total 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 evaporated in a rotary evaporator (iGene Labserve, New Delhi, India) to afford a blackish mass. The completely evaporated extract was subsequently collected in 2 ml eppendorf tube and stored at 4°C for investigations.

2.4 Zebrafish embryo collection

The cytotoxicity of *A. augustum* seed extract was examined in zebrafish embryos in accordance with the Organization for Economic Cooperation and Development [15]. Zebrafish embryos were collected from the same spawn of eggs and selected under a light microscope at 1 h post fertilization (hpf). Eggs were washed with sterilized water after fertilization and reared at 28°C in E3 media [16]. All eggs were collected and maintained via the method of Ruokonen et al. [17].

2.5 Detection of phytochemicals

Phytochemical detection was performed on *A. augustum* seed extracts in methanol via standard processes as previously reported [18–21]. To detect different phytochemicals, 5 g of crude extract was added separately for each test.

2.6 Quantitative analysis of phytochemicals

The detected phytochemicals in *A. augustum* seed extract were measured via previous techniques, as described by Hazra et al. [22] and Longbap et al. [19]. To quantify the detected phytochemicals, 10 g of extract was added individually for each test.

2.7 Antioxidant activity

The free radical scavenging ability of *A. augustum* seed extract was evaluated via a previously described method [23]. Briefly, 3 ml of the reaction mixture was prepared with 1 ml of 0.3 mM DPPH, 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:

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

Here, A_0 represents the absorbance of the control sample, and A_1 represents the absorbance of the sample. All the experiments were replicated three times. The data were calculated as the average percentage of antiradical action.

2.8 Cytotoxicity assay

The cytotoxicity of *A. augustum* seed extract on zebrafish embryos was studied via the Souza et al. [24] method. 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.

2.9 Statistical analysis

The mean values were calculated from three different experiments, and the data are presented as the standard error of the mean (mean ± SEM), using one-way ANOVA by SPSS software version 17 (SPSS, Chicago, IL, USA). Duncan's multiple range test (DMRT) was used to determine the significance of differences between the treatments at $p < 0.05$.

3 Results

3.1 Phytochemical screening

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. The data are presented in Table 1.

Table 1
Phytochemical screening of methanol extracts of *Abroma augustum* seeds. Here, (+) = present, (-) = not present

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	-

3.2 Quantification of phytochemicals

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. The data are presented in Fig. 1.

3.3 Antioxidant activity

The methanol extract of *A. augustum* seeds showed the highest scavenging of $65.30 \pm 0.9\%$ DPPH, whereas the standard BHT value was $80.85 \pm 1.0\%$ at a concentration of 200 $\mu\text{g/ml}$. In addition, the percentages of DPPH inhibition by the seed extract and BHT were $20.95 \pm 1.5\%$ and $46.98 \pm 1.5\%$, respectively, at a concentration of 50 $\mu\text{g/ml}$. The IC_{50} values of the seed extract and BHT were 105.57 ± 1.19 and 88.89 ± 1.0 $\mu\text{g/ml}$, respectively. The details are presented in Fig. 2.

3.4 Cytotoxic effects of the seed extracts on zebrafish embryos

At 25 hpf, the application of 100 $\mu\text{g/ml}$ 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 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. The details are presented in Fig. 3.

4 Discussion

In the recent era, many plants have been used to produce phytochemicals for drug development in the medical and pharmaceutical sciences. Phytochemicals protect plants, humans and animals from several diseases [25]. In the present study, several important phytochemicals, such as alkaloids, carbohydrates, flavonoids, glycosides, polyphenols and tannins, were detected in *Abroma augustum* seed extract. Previously, similar phytochemicals were detected by Das et al. [26] in leaves and roots and Hazra et al. [22] in the stem bark of *A. agusta* plants. These findings support our present results. In contrast, amount of total phytocompounds may be somewhat different from present findings due to the quantity of secondary metabolite, topographical disparity, period of the investigations, or extraction technique [27].

The present study revealed high contents of total phenols (9.11%), polyphenols (8.76%), alkaloids (6.33%) and flavonoids (5.09%) in the seed extract of *A. agusta*. Khatoon et al. [28] stated related concentrations of total phenols, flavonoids, saponins and alkaloids in *Tabernae montana* root extracts. Longbap et al. [19] determined significant levels of phytochemicals in *Hannoa undulata* leaf extracts. Alfalluos et al. [29] described similar findings for Retama extract. In contrast, Hazra et al. [22] reported low concentrations of total phenolics ($2.21 \pm 0.39\%$), flavonoids ($0.51 \pm 0.02\%$), tannins ($0.88 \pm 0.02\%$) and alkaloids ($1.38 \pm 0.37\%$) in *Abroma augusta* stem bark extracts.

The present study revealed $65.30 \pm 0.9\%$ DPPH inhibition, and the IC_{50} value was 105.57 ± 1.19 for the 200 $\mu\text{g/mL}$ seed extract. Yadav and Singh [30] reported similar antioxidant effects by leaf extracts of *A. augusta*. Similar data were observed by Ismail et al. [31] in *Andrographis paniculata* leaves and *Cinnamon zeylanicum* bark extracts. In contrast, Sunitha et al. [9] reported an IC_{50} value of 790 ± 3.6 $\mu\text{g/mL}$ for the Malaysian *A. augusta* plant extract. Ivy et al. [12] also reported an IC_{50} value of 36.70 ± 0.32 $\mu\text{g/mL}$ crude extract of *A. augusta* leaves. These data support our present findings.

In the cytotoxicity experiments, 100 $\mu\text{g/mL}$ seed extract-treated zebrafish embryos at 25, 50 and 96 hpf presented abnormal growth and development. Makkar et al. [32] reported the toxicity of dental bioceramics in embryonic zebrafish. Shaikh et al. [33] reported a little detached tails of zebrafish embryos treated with *Ficus glomerata* aqueous extract at 24 hpf. 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 [34]. *Curcuma longa* extract has teratogenic effects, body deformities, and enlarged yolk sacs at 96 hpf in zebrafish embryos [35]. Safflower caused pericardial and yolk-sac edema at 96 hpf in zebrafish embryos at a concentration of 345.6 mg/L [36]. Aksakal and Abdulkadir [37] found similar effect at 96 hpf using 1.6–2.4 mg/ml of penconazole was

used. In contrast, natural cosmetic ingredient mixtures have shown no cytotoxicity or toxicity to zebrafish embryos at high concentrations [38]. Wibowo et al. [39] also stated no toxicity on zebrafish embryos treated by pomegranate peel extract with $196.037 \pm 9.2 \mu\text{g/mL}$ at 96 hpf. Moreover, some chemical substances showed different effects on developmental stages of zebrafish embryos [40].

5 Conclusions

Abroma augustum seed looks to be a hopeful source of biologically potential composites that might be able to controlling human diseases difficulties. Moreover, the seeds of this plant could be applied for antioxidant activity. The methanol extract of *A. augustum* seeds contains some promising phytocompounds containing significant antioxidant potency and cytotoxic effects on zebrafish embryos. Further investigation is needed to confirm the cytotoxicity that may be helpful for novel drug designs in the pharmacological industries.

Abbreviations

DPPH 1,1-diphenyl-2-picrylhydrazyl radical

BHT Tert-butyl-1-hydroxytoluene

HPLC High-performance liquid chromatography

DMSO Dimethyl sulfoxide

hpf Hours post fertilization

IC₅₀ Half maximal inhibitory concentration

Declarations

Author contributions H.B.S. and A.K performed material collection, methodology, investigation and wrote the main manuscript. M.E.H and B.S. done formal analysis, data curation Table and Figures preparation. M.F.H. ensured conceptualization, manuscript revision, editing and supervision. All authors reviewed the manuscript.

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Plant guidelines The collection of plant material complies with relevant institutional, national, and international guidelines and legislation. No approval is needed from the authority in Bangladesh to

collect *Abroma augustum* seeds for research purposes.

Animal guidelines The procedures of the investigations were designed according to the techniques for the use of laboratory animals and precautions approved by the Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee, Institute of Biological Sciences, University of Rajshahi, Bangladesh (Memo No. 255(20)/420/IAMEBBC/IBSc).

Data availability statement The datasets used during the current study are available from the corresponding author on reasonable request.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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Figures

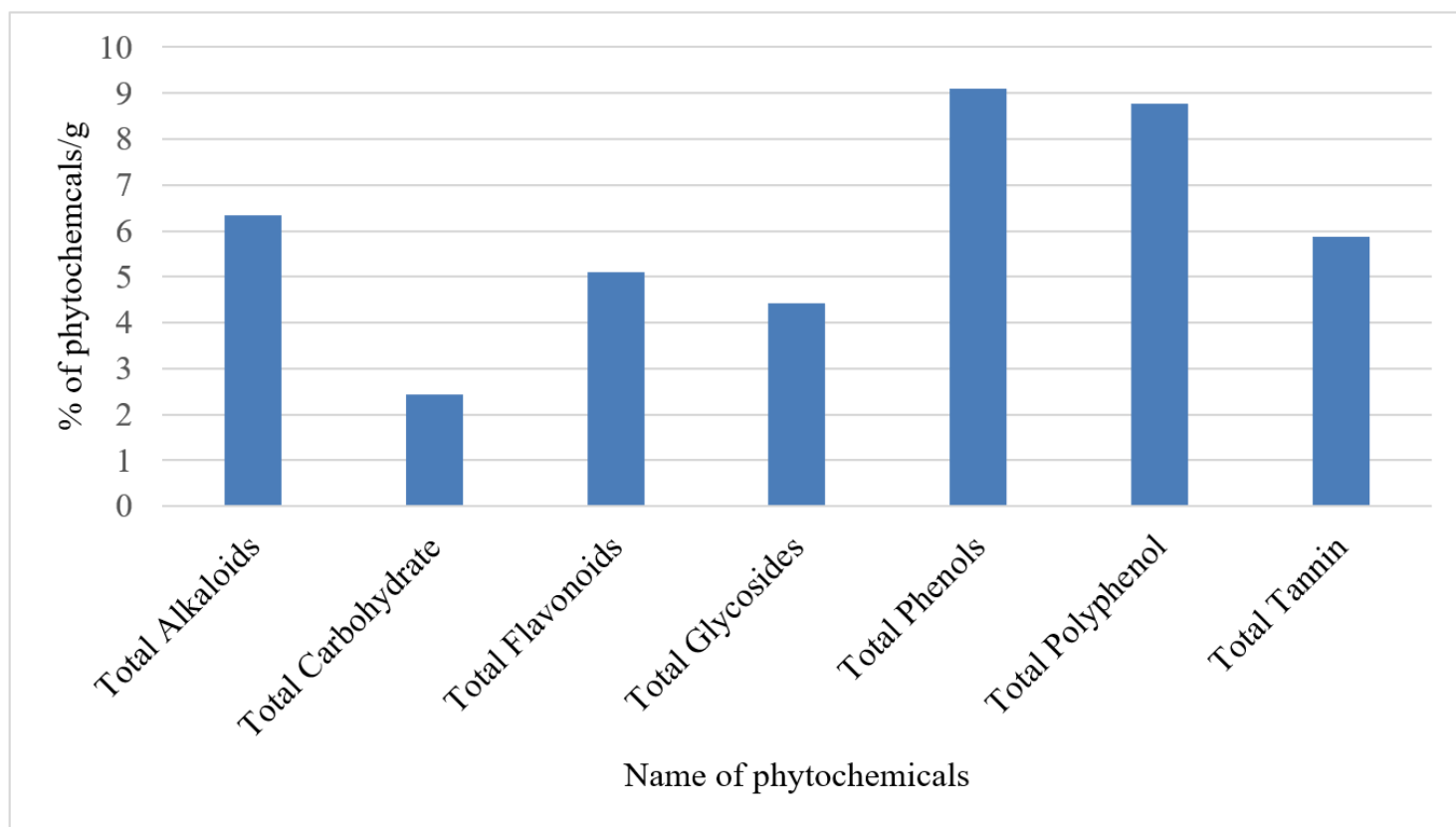


Figure 1

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

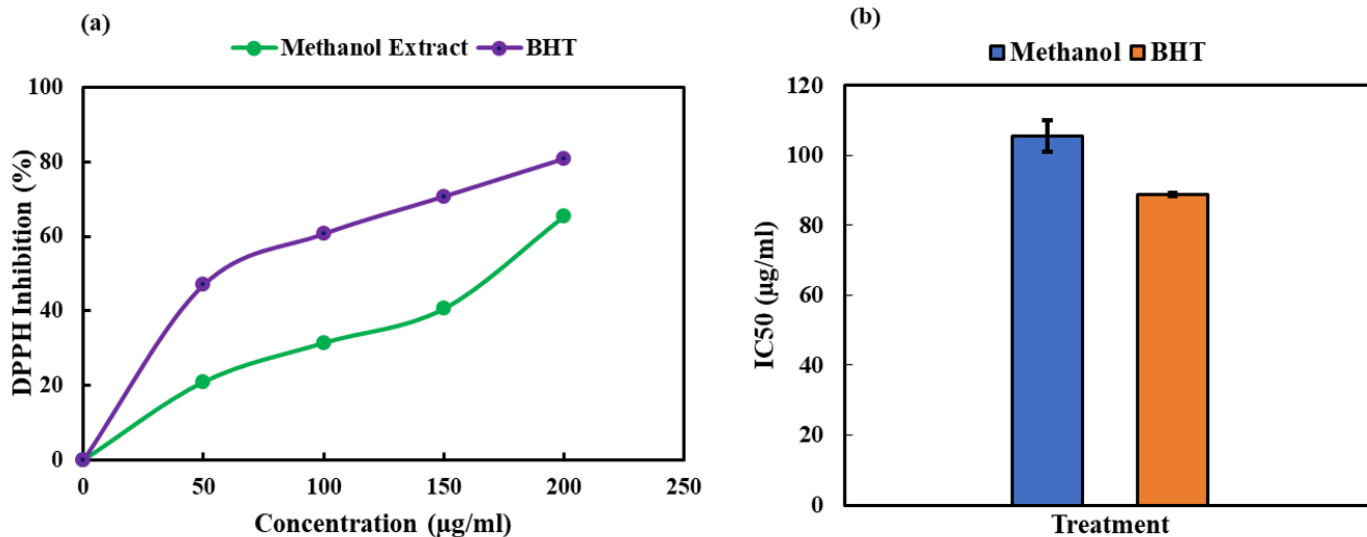


Figure 2

Antioxidant effects of *A. augustum* seeds, where (a) DPPH inhibition percentage and (b) IC₅₀ (concentration that inhibits 50%) value.

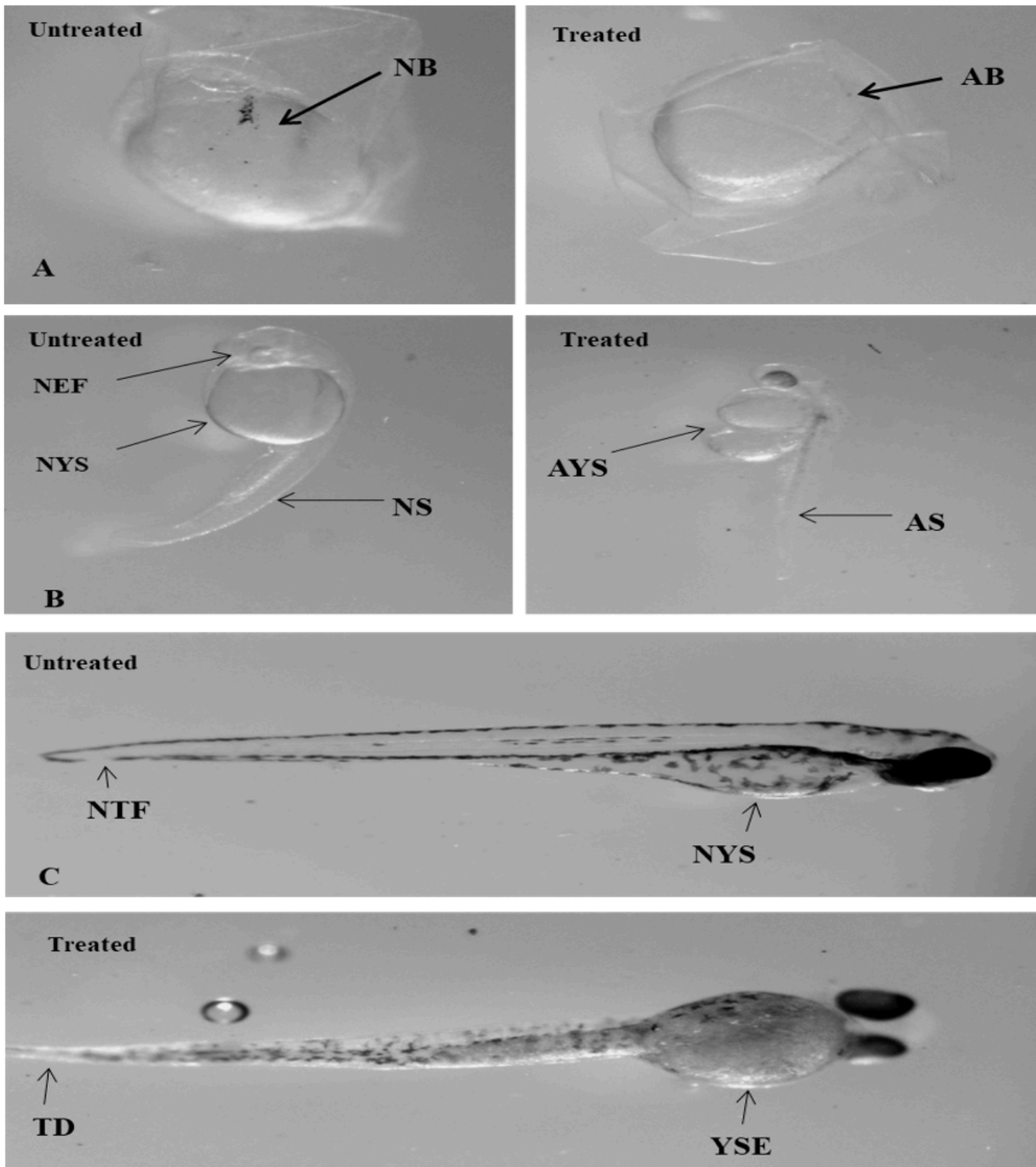


Figure 3

Zebrafish embryo development in the untreated and seed extract-treated groups: (A) 25 hpf, (B) 50 hpf and (C) 96 hpf. Here, NB represents a normal blastomere, AB represents a normal blastomere, NEF represents normal eye formation, NYS represents a normal yolk sac, NS represents a normal spine, AEF represents abnormal eye formation, AYS represents an abnormal yolk sac, AS represents an abnormal

spine, NTF represents normal tail formation, NYS represents a normal yolk sac, TD represents tail deformation and YSE represents yolk sac edema.