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Original article

A comparative study of biological potentiality and EAC cell growth inhibition activity of *Phyllanthus acidus* (L.) fruit pulp and seed in Bangladesh

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ABSTRACT

Medicinal plant-derived bioactive compounds have recently gained more interest in biological research as an important source of novel drug candidates. *Phyllanthus acidus* (L.) is a widely distributed herbal medicinal plant naturally used in Ayurvedic medicine in Bangladesh. The present study focused on exploring the biological potential as well as the inhibitory effect of EAC cell growth with a comparative analysis between *Phyllanthus acidus* fruit pulp and seed. Crude methanol extract of *P. acidus* (MEPA) fruit pulp and seed was assessed as DPPH and NO free radical scavengers. While Brine Shrimp lethality bioassay, the standard protocol of phytochemical screening and hemagglutination assay were performed successively to determine the toxic effect on normal cells, the identification of some crucial phytochemicals, and the existence of lectin protein. EAC (Ehrlich's Ascites Carcinoma) cell growth inhibition was determined by hemocytometer and morphological changes of EAC cells were observed by a fluorescence microscope using Swiss albino mice. The IC_{50} value of MEPA fruit pulp and seed was obtained as 57.159 μg/ml and 288.743 μg/ml respectively where minimal toxic effects on Brine Shrimp nauplii demonstrates that it is a good source of natural antioxidant compounds. Again, MEPA fruit pulp and seed-mediated effective agglutination of mouse blood erythrocyte strongly support the presence of lectin protein. Furthermore, MEPA fruit pulp and seed extract-treated EAC cells showed 65.71% and 28.57% growth inhibition respectively. The fluorescent microscopic examination of EAC cells treated with MEPA fruit pulp has shown more remarkable structural changes in the nucleus than that of seed. Based on the above findings, the present study reveals that MEPA fruit pulp can be considered as a novel biological candidate for the treatment of fatal diseases shortly.

1. Introduction

Medicinal plants are the most reliable sources for exploring the biological activities of natural products. They also have a significant number of applications in the pharmaceutical, ingredients, insecticides, dyes, flavorings, and fragrance sectors (Vuorela et al., 2012). Nowadays, researchers have introduced medicinal plant-derived natural products as an alternative to synthetic drugs due to the side effects, high treatment costs, and insufficient drug supply. The World Health Organization (WHO) reports that around 80% of the World's population relies on conventional herbal medicines for their primary health care considered to be very safe due to minimal or no side effects (Farnsworth et al., 1985; Kim et al., 2017).

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Oxygen is a crucial organic component of aerobic life, but its reduced form, such as ROS (reactive oxygen species), is one of the most toxic substances responsible for causing several diseases such as inflammation, carcinogenesis, atherosclerosis, neurodegenerative disorders, cardiovascular dysfunction, etc. (Stankovic et al., 2011). Superoxide anion and hydroxyl radicals are the two most common examples of oxidation–reduction (redox) reactions produced as a by-product (Halliwell, 2006; Saeed et al., 2014). ROS homeostasis has reached the balance between ROS development and ROS scavenging in cellular organisms by antioxidant compounds. However, antioxidant substances may neutralize or scavenge free radicals that are typically involved in oxidative stress-mediated cellular damage (Dakah et al., 2014; Yıldırım et al., 2000). A recent study reported that antioxidant components including phenols, flavonoids, carotenoids, alkaloids, terpenoids have been used for several biological activities such as antioxidative, anti-inflammatory, anti-hepatotoxic, antimicrobial, and anti-carcinogenic effects (Brooks et al., 2020; Figueroa et al., 2014; Kumarappan et al., 2012). Moreover, Plant lectins, a unique group of proteins and glycoproteins, have been found to have potential anti-carcinogenic effects (Kabir et al., 2015). It is well documented that traditional medicine is one of the primary health care systems for treating disorders in many other developing countries, such as Bangladesh (Dubey et al., 2004; Moniruzzaman et al., 2015). Again, Bangladeshi people have traditional medical practice as an integral part of their culture due to the availability of medicinal plants in that locality. Therefore, the assessment of local flora for the detection of different biological potentials is the first step necessary for the isolation and characterization of drug-leading bioactive compounds.

Phyllanthus acidus(L.) Skeels, belonging to Phyllanthaceae family (Brooks et al., 2020), is a widely distributed herbal medicinal plant, particularly grown in Bangladesh and the Southern part of India (Jain and Singhai, 2011). This plant is locally known as Amla or Orbori in Bangladesh (Pasha and Uddin, 2019) and Otaheite gooseberry or star gooseberry in India (Brooks et al., 2020), which is typically an intermediary between shrubs and trees, reaching 2 to 9 m (6½ to 30 ft) high. *P. acidus* fruits have already been identified as an abundant source of ascorbic acid, fibers, phenolics, flavonoids, and carotenes while seeds are used as cathartic (Brooks et al., 2020; Unander et al., 1990). Many parts of this plant have historically been used in the treatment of various vitiated conditions such as jaundice, bronchitis, constipation, diarrhea, biliousness, urinary concretions, and piles (Kirtikar and Basu, 1987; Tan et al., 2020). Also, the plant is essential to increase eyesight, memory, and relieve pain from cough, psoriasis, skin disorders, and sudorific (Banik et al., 2010; Devi and Paul, 2011).

Keeping in view the medicinal benefit of *P. acidus* (L.), the current research has been designed to test the crude methanol extract of *P. acidus* (L.) (MEPA) fruit pulp and seed-mediated biological properties i.e. antioxidant activity, cytotoxicity, phytochemical screening, hemagglutination activity, and evaluation of EAC cell growth inhibitory effect with morphological changes of EAC cell nuclei. A comparative analysis of these two extracts was also carried out based on the results of the biological assays mentioned above.

2. Materials and methods

2.1. List of chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), DAPI fluorescent dye and 0.4% trypan blue dye (Sigma chemical company, USA), Ethanol and Methanol (Sigma chemical company, USA), Ascorbic acid (Merck, Germany), De-ionized water, Sodium chloride (NaCl), Mayer's reagent, Hydrochloric acid (1.5 %v/v), Wagner's reagent, Chloroform, Concentrated Sulphuric acid, Fehling's solution, 5% $FeCl_3$ solution, Ninhydrin solution, Hemagglutination or PBS buffer (20 Mm Tris-HCl buffer, 1% NaCl, 10 mM $CaCl_2$, pH 7.8), Artemia salina leach (Brine eggs), Sea salt (NaCl) (Roth Chemicals, India).

2.2. Collection of plant samples

Fresh *P. acidus* (L.) fruits were collected from the germplasm bank of Bangladesh Agricultural Research Institute (BARI), Rajshahi, Bangladesh in October 2017 during fruiting. The plant part was kindly provided by Dr. Alim Uddin, Chief Scientific Officer of BARI. Afterward, fruit samples were authenticated by the taxonomist of the Department of Botany, University of Rajshahi, Bangladesh.

2.3. Preparation of crude methanol extract

The crude methanol extract of desired samples was prepared following the method described by Olayaki et al. (2015) with slight modification. At first, collected fruit samples were sterilized with distilled water and then fruit samples (flesh) were cut into small pieces. Then seeds were isolated from flesh and both samples were dried in the drier at 30° C for 7 days. After that dried samples were grinded to make a coarse powder and preserved at room temperature. 100 g of flesh powder and 20 g of seed powder was then extracted with 100% methanol and shacked at 140 rpm for 2 h separately. Then the shacked samples were sonicated by using a sonicator and shaken overnight at 140 rpm. Again the samples were filtered by vacuum filter and then the filtrates were evaporated by using a rotary evaporator at 35 °C temperature. Finally, dark brownish-green semisolid extracts in both cases were obtained and stored at −20 °C for further experiments.

2.4. Determination of antioxidant activity

a) DPPH free radical scavenging assay

DPPH was used to evaluate the free radical scavenging activity of crude methanol extract of *P. acidus* (MEPA) fruit pulp and seed following DPPH assay based on the method described by Parejo et al. (2000) with a slight modification. At first, the stock solution of DPPH was prepared by dissolving 24 mg of DPPH into 100 ml methanol and stored at −20℃ until use. Then, 1 mg of MEPA fruit pulp and seed were dissolved into 1 ml distilled water separately to prepare the dose for treatment. Afterward, MEPA fruit pulp, seed, and Ascorbic acid (Aa) mediated stock solutions were taken in different test tubes at different concentrations ranging from 12.5 to 200 μg/ml. After that 1.5 ml of DPPH solution was added to each test tube. Then the reaction mixture of each test tube was shaken vigorously and incubated at room temperature for 30 min in a dark place to complete the reaction. After incubation, the absorbance of decolorized DPPH was measured at 517 nm using a spectrophotometer against a blank solution (Methanol). A typical standard solution contained all reagents except desired plant extract or Ascorbic acid was considered as control. The percentage (%) of inhibition activity was calculated from the following equation;

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

Where A_0 is the absorbance of the control (without extract) and A_1 is the absorbance of extract.

Then the scavenging percentages of DPPH free radical for different concentrations were plotted against concentration and finally, the IC_{50} value was calculated from the equation of the graph.

b) Determination of nitric oxide (NO) scavenging assay

Nitric oxide free radical scavenging activity of methanol extract of *P. acidus* fruit pulp and seed was determined according to the method described by Marcocci et al. (1994) with some modifications. A volume of 2 ml of 10 mM sodium nitroprusside dissolved in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of desired fruit pulp and seed extract at various concentrations in different test tubes. The reaction mixture was then incubated at 25 °C. After 150 min of incubation, the incubated mixtures were then mixed with 0.5 ml of Griess reagent (0.33% sulfanilic acid and 0.1% NED in 20% glacial acetic acid). After that, the mixture was again incubated at room temperature for 30 min and then the absorbance of each reaction mixture was measured at 550 nm through transferring into a cuvette. Ascorbic acid was used as a reference standard. The amount of nitric oxide free radical inhibition was calculated using the following equation:

Percentage (%) of inhibition of NO free radical = $[\{A_0 - A\}/A_0] \times 100$

Where A_0 is the absorbance of blank control (NO radical solution without test sample) and A is the absorbance of the test sample. Finally, the percentage (%) of inhibition was plotted.

2.5. Determination of cytotoxicity through Brine Shrimp lethality bioassay

Cytotoxic activity was done by Brine Shrimp lethality bioassay following the method of Islam et al. (2018a), Meyer et al. (1982) with *Artemia salina* nauplii. For this experiment, at first Brine Shrimps were hatched in a 1L beaker filled with NaCl solution at the concentration of 38 g/L. Then 10 test tubes were taken and filled with 25 ml NaCl solution in each test tube. After that 20 nauplii were taken in each test tube with the help of a micropipette and then crude MEPA fruit pulp and seed were added into the test tubes at different doses. Finally, the test tubes were incubated at room temperature for 24 h and the 50% lethal concentration (LC_{50}) value was estimated by using a regression line obtained from the plotting of mortality percentages against different concentrations.

2.6. Determination of phytoconstituents

Preliminary phytochemical analysis of crude MEPA fruit pulp and seed were conducted based on the following standard protocols described by Miah et al. (2020), Bhandari et al. (2017), and Senguttuvan et al. (2014) to detect the presence of phytoconstituents including alkaloids, phenols, flavonoids, steroids, saponins, tannins, glycosides, terpenoids, proteins, and carbohydrates in studied materials.

2.6.1. Test for alkaloids

2.6.1.1. Mayer's test. For this test, a few drops of Mayer's reagent and 2 mg of respective MEPA fruit pulp and seed extract were mixed separately. The formation of white or pale yellow precipitate was indicated the presence of alkaloids (Bhandari et al., 2017).

2.6.1.2. Wagner's test. In case of Wagner's test, at first hydrochloric acid (1.5 %v/v) was added to 2 mg of crude methanol extract of *P. acidus* (L.) (MEPA) fruit pulp and seed until the media was acidic and then a few drops of Wagner's reagent were added. Yellow or brown precipitation was indicated the presence of alkaloids (Bhandari et al., 2017).

2.6.2. Test for flavonoids

For the flavonoid test, 1 mg of respective plant extracts were first dissolved into distilled water in different testubes and then concentrated HCl was added from the side of testubes. The presence of the flavonoid was confirmed by red color formation (Zhishen et al., 1999).

2.6.3. Test for steroids

Firstly, 1 mg of MEPA fruit pulp and seed extract was dissolved into 10 ml of chloroform in two testubes separately for the detection of steroids. Then an equal volume of concentrated Sulfuric acid was added by the side of testubes. The presence of steroids was indicated by the formation of red color in the upper layer and the turn of Sulfuric acid layer into yellow color with green fluorescence (Senguttuvan et al., 2014).

2.6.4. Test for terpenoids

While for the detection of terpenoid, 5 mg extract was taken from both samples of *P. acidus* and mixed with 2 ml of chloroform and then 3 ml of concentrated Sulfuric acid were added to form a layer. Reddish-brown colored precipitate formation at the interface was indicated the presence of terpenoids (Uddin et al., 2012).

2.6.5. Test for glycosides

A small amount of MEPA fruit pulp and seed were taken first and then dissolved into distilled water to identify glycosides. After that Fehling's solution was added within the dissolved watery extract in both cases within separate testubes where the brick-red precipitate formation was indicated the presence of glycosides in the experimental sample (Nisar et al., 2011).

2.6.6. Test for saponins

In case of the saponin test, 5 ml of aqueous extract in both cases was strongly shaken in different testubes. The presence of persistent frothing upon warming for 2–3 min was indicated the evidence of saponins presence in studied materials (Bhandari et al., 2017).

2.6.7. Test for tannins

For detecting the presence of tannins, 1 mg extract in both cases was taken in different test tubes and continuously stirred with distilled water. Then 5% $FeCl_3$ solution was added in two test tubes separately. The absence of blue-black color precipitation was directly indicated the absence of tannins (Siddhuraju and Manian, 2007).

2.6.8. Test for phenols

First, 1 mg MEPA fruit pulp and seed extract was dissolved into 1 ml of distilled water in different test tubes and after that 5% $FeCl_3$ solution was added into two test tubes separately. The presence of phenolic compounds in both extracts was detected based on the formation of a dark green color (Siddhuraju and Becker, 2003).

2.6.9. Test for proteins

For the detection of proteins, 2–3 mg of MEPA fruit pulp and seed extract dissolved in distilled water were taken in two test tubes and then 3–4 drops of ninhydrin solution were added and finally kept on a water bath to give heat. The appearance of purple or violet color was indicated the presence of protein (Bhandari et al., 2017).

2.6.10. Benedict's test for carbohydrates

Again, for the identification of carbohydrates, 0.5 mg extract from both fruit pulp and seed of *P. acidus* (L.) were taken firstly in different test tubes, and then 1 ml distilled water was added into the test tubes separately. Finally, 5–8 drops of Fehling's solution (hot) were added. The presence of brick-red precipitate formation was indicated the presence of carbohydrates (Bhandari et al., 2017).

2.7. Determination of lectin protein activity

Haemagglutination is a specific form of agglutination which is involved to agglutinate the red blood cells (RBCs) in the presence of lectin proteins. The presence of lectin activity of crude MEPA fruit pulp and seed was estimated following hemagglutination assay described by the method of Islam et al. (2018a). At first, 1 mg crude extract from both samples was weighted, and then working extracts were prepared through centrifugation at 8000 rpm for 15 min. Again, 2% suspension (w/v) of RBCs was prepared with hemagglutination buffer through the centrifugation of collected mouse blood. After that hemagglutination reaction was performed in 96-well microtiter U-bottomed plates described by Hasan et al. (2019) with a slight modification. For performing this reaction, first of all, 50 μl of hemagglutination buffer was taken in every well of titer-plate, and then 50 μl prepared extracts of both samples were added to the first well of titer plate and serially diluted into the successive wells with PBS buffer (pH 7.4). Finally, an aliquot of 50 μl of 2% RBCs suspension was added to each well. PBS buffer along with 2% RBCs suspension without the extract was considered as control. Then this titer plate was shaken by a micro-shaker at 300 rpm for 20 min and after shaking placed on a table under room temperature. After 15–20 min, the agglutination of blood erythrocytes was observed and agglutination activity was assessed based on the rough granules within the titer plate.

2.8. Experimental animal care with ethical permission

The current experimental technique was carried out after getting the permission of the Institutional Ethics Committee for the experimentations on animal, human, microbes, and living natural sources (225/320-IAMEBBC/IBSc), Institute of Biological Sciences, University of Rajshahi, Bangladesh.

For conducting such kind of experiment, female Swiss albino mice (weighing between 15 and 20 g) were collected from the Department of Pharmacy, Rajshahi University, Rajshahi. Then the mice were grouped and housed in plastic cages with not more than five animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C; humidity 55 ± 5%) with 12 dark/light cycles. Standard dry pellet diet (collected from ICDDR, B) and water *ad libitum* were supplied for their survival. Besides, the mice were adapted in our laboratory conditions for 7 days before starting the final experiment.

2.9. Propagation and inoculation of EAC cell into normal Swiss albino mice

Ehrlich's Ascites Carcinoma (EAC) cells are commonly used in the field of ascitic tumor research worldwide due to the fastest-growing capacity and the easiest transferrable property (Rajkapoor et al., 2007; Zahan et al., 2019). Initial inoculum of EAC cells was provided by the Indian Institute of Chemical Biology (IICB), Kolkata, India. Then the continuous propagation of EAC cells into normal mice peritoneum was properly maintained in the laboratory biweekly via intraperitoneal (i. p.) injections of 1×10^6 to 2×10^6 cells suspended in 0.3 ml of the sterile saline solution following the protocol of Alam et al. (2016). In brief, first in vivo propagated EAC cells were drawn out from ascitic fluid of tumor-bearing Swiss albino mice at the respective log-phases of tumor cells. Then the freshly drawn fluid was diluted with normal saline (1% NaCl solution) and the number of tumor cells was adjusted to approximately 1×10^6 cells/ml by counting cell numbers with the help of a hemocytometer. The viability of tumor cells was observed by trypan blue dye (0.4%) exclusion assay. Finally, 100 μl tumor cell suspension was injected into each mouse intraperitoneally (i. p.) and proper aseptic condition was maintained throughout the process.

2.10. Determination of EAC cell growth inhibition

EAC cell growth inhibition was determined based on the technique of Kabir et al.(2015) with slight modification. To evaluate the cell growth inhibition, three groups of Swiss albino mice (5 in each group) weighting 20–25 g were used where 1×10^6 EAC cells/ml were inoculated intraperitoneally (i. p.) on each group of mice on the day "0". Hence, group one mice was used as positive control while MEPA fruit pulp and seed extract-treated mice were considered as group 2 and 3 respectively. MEPA fruit pulp and seed extract treatments (100 mg/kg body weight/day) were started after 24 h of EAC cell inoculation and continued for six days. Then mice from each group were sacrificed on day seven and intraperitoneal EAC cells were harvested.

After the collection of EAC cells, they were counted by a hemocytometer. The total number of cells in MEPA fruit pulp and seed extract-treated groups were then compared with the control group. Following formula was used to calculate the percentage of cell growth inhibition:

% Cell growth inhibition =(1-Tw/Cw) × 100

Where Tw = Mean of the number of tumor cells of the treated group of mice and Cw = mean of the number of tumor cells of the control mice group.

2.11. Evaluation of morphological changes of EAC cells

To observe the morphological changes of the EAC cell, the ascitic fluid obtained from treated mice of each group were stained with DAPI fluorescent dye and kept in dark condition at 37 °C for 15 min. After that continuous washing and re-suspension strategy were performed with PBS buffer to take the visual images of EAC cell morphology under the fluorescence microscope (Olympus ix71, Korea). Next, 5 μl supernatant was placed on a microscopic slide and the whole technique was done following the method of Miah et al. (2020) and Islam et al. (2015).

2.12. Statistical analysis

All results are expressed as mean ± SEM and carried out in triplicates for the determination of significant differences between the mean of two test samples. Hence, all data were assessed by one-way ANOVA followed by Duncan's multiple range test (DMRT) and *t*-test using SPSS software 16 where $P < 0.05$ was considered as statistically significant and all of the graphs prepared by using GraphPad Prism 9 software.

3. Results

3.1. Evaluation of antioxidant activity

3.1.1. DPPH free radical scavenging assay

DPPH is a stable free radical and the color change of DPPH is proportional to the decrease of absorbance number that indicates significant free radical scavenging activity of test samples. Again, the higher DPPH scavenging activity indicates a higher antioxidant activity. The IC_{50} value for scavenging of DPPH free radical by MEPA fruit pulp and seed was found to be 57.159 ± 0.210 μg/ml and 288.743 ± 0.194 μg/ml respectively compared to the IC_{50} value of ascorbic acid which was approximately 9.888 ± 0.150 μg/ml at different concentrations. The highest scavenging activity of 88.39% for MEPA fruit pulp and 31.60% for MEPA seed was estimated at the concentration of 200 μg/ml while ascorbic acid exhib-

ited the highest inhibition of 92.14% at the same concentration (Supplementary Table S1). The percentage (%) of scavenging activity with different concentrations and IC_{50} value of MEPA fruit pulp and seed compared to ascorbic acid are shown in Fig. 1.

3.1.2. Nitric oxide (NO) scavenging assay

The reduction capability of NO was determined by the decrease in absorbance than control at 550 nm induced by antioxidant compounds. NO free radical inhibition activity of MEPA fruit pulp, seed, and Ascorbic acid (standard) is shown in Fig. 2 at different concentrations.

3.2. Determination of cytotoxicity

The lethal concentration value(LC_{50}) of test samples was calculated from the regression equation obtained by plotting the sample concentration against the mortality percentage of Brine Shrimp nauplii. The LC_{50} value of MEPA fruit pulp and seed was obtained as 485.005 μg/ml and 665.393 μg/ml respectively which is shown in Fig. 3.

3.3. Determination of phytoconstituents

On preliminary phytochemical analysis, the methanol extract of *P. acidus* (MEPA) fruit pulp shows the presence of alkaloids, phenols, flavonoids, terpenoids, glycosides, proteins, carbohydrates where steroids, saponins, and tannins phytochemicals are absent. On the other hand, MEPA seed demonstrates the presence of alkaloids, phenols, glycosides, saponins, protein, and the absence of flavonoids, steroids, terpenoids, tannins, and carbohydrates. Hence, the presence of phytochemicals is shown by (+) sign and the absence of phytochemicals is shown by (-) sign. Phytoconstituent determination results are shown in Table 1.

3.4. Assessment of lectin protein activity

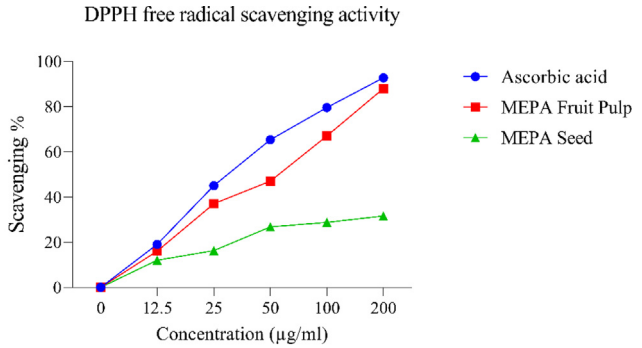
The presence of lectin protein which is ubiquitously found in plants and other organisms was determined by the hemagglutination assay. This experiment exhibits that MEPA fruit pulp and seed have high agglutination activity against 2% RBCs suspensions at the lowest concentration of 31.25 μg/well where the negative control wells did not show any kind of agglutination activity. The agglutination activity of MEPA fruit pulp and seed is demonstrated in Fig. 4**.**

3.5. Determination of EAC cell growth inhibition

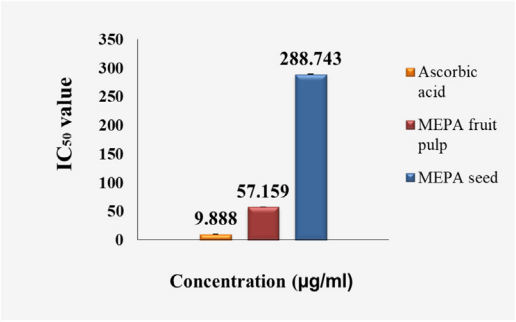
The number of viable EAC cells of the control group was found to be $7.0x10^6$cells/ml, where in case of the fruit pulp extract-treated group was found to be $2.4x10^6$cells/ml and the seed extract-treated group was found to be $5.0x10^6$cells/ml shown in Fig. 5. Hence, 65.71% (B) and 28.57% (C) EAC cell growth inhibition were determined in both cases respectively shown in Table 2.

3.6. Observation of morphological changes of EAC cell under the fluorescence microscope

Morphological changes of EAC cells collected from both control mice and mice treated with MEPA fruit pulp and seed extract were examined by DAPI staining assay after sacrificing on 7^{th}day. The result of EAC cell observation from control mice and both extracts treated mice under a fluorescence microscope is manifested in Fig. 6. Under the microscopic observation, EAC cells nuclei from the control group were found as round, regular, and homogeneously stained with DAPI (1 mg/1.6 ml) shown in Fig. 6**(A),** Whereas, some of the EAC cells treated by MEPA fruit pulp and seed were shown fragmented nuclei where more fragmented nuclei were observed in case of fruit pulp than seed shown in Fig. 6 **(B) & (C).**



(A)



(B)

Fig. 1. The antioxidant activity of MEPA fruit pulp and seed. (A) DPPH free radical scavenging % of MEPA fruit pulp and seed extract at different concentrations along with Ascorbic acid standard. (B) Comparison of IC_{50} value of MEPA fruit pulp and seed extracts with standard Ascorbic acid at different concentrations. Data are expressed as mean ± SEM (n = 3), where significance level was considered at $P < 0.05$.

4. Discussion

A large number of drugs have been developed recently from plant-derived natural compounds to treat severe disorders without any toxic effect (Newman and Cragg, 2016) and *Phyllanthus acidus* is considered a good source of medicine due to its extensive application in aspect of the treatment of inflammation and pain, oxidative stress-related disorders, hepatic injury, and gonorrhea (Chakraborty et al., 2012). Therefore, it is decided to check the biological potentiality and EAC cell growth inhibition activity of this plant part specifically fruit pulp and seed which is available in Bangladesh round the year.

The antioxidant activity of *P. acidus* fruit pulp and seed extract has been tested following two widely used methods such as DPPH and NO scavenging assay. DPPH free radical scavenging assay is a rapid, simple, and inexpensive method widely used to test the ability of antioxidant compounds found in various parts of plant samples (Lee et al., 2007). However, previous studies on a few species of this genus have been already shown promising antioxidant activity(Andrianto et al., 2017; Habib et al., 2011). The result of this test typically indicates that MEPA fruit pulp has a strong ability to scavenge DPPH free radical than MEPA seed when compared with the IC_{50} value of Ascorbic acid standard. The IC_{50} value of MEPA fruit pulp and seed was found to be 57.159 μg/ml and 288.743 μg/ml respectively while the IC_{50} value of Ascorbic acid

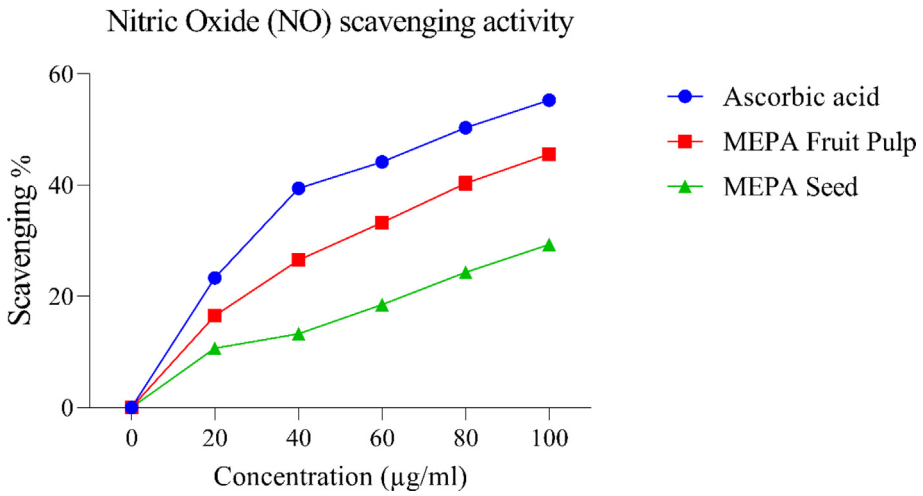


Fig. 2. Nitric Oxide (NO) Scavenging activity. NO free radical scavenging % of MEPA fruit pulp and seed along with standard Ascorbic acid at different concentrations. Results are expressed as mean ± SEM (n = 3), where significance level was considered at P < 0.05.

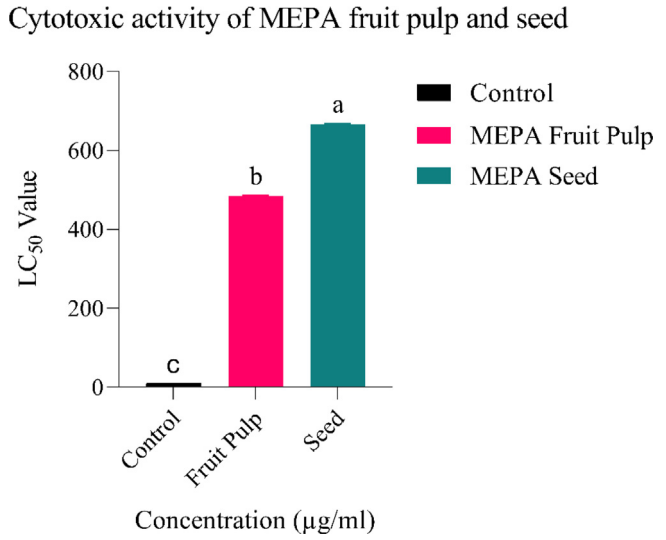


Fig. 3. Cytotoxic activity of MEPA fruit pulp and seed at different concentrations. LC_{50} values of MEPA fruit pulp and seed are compared with control group which typically indicates that both are significantly different from each other, where significance level was set at P < 0.05.

Table 1
Results of phytochemical screening of MEPA fruit pulp and seed.

Phytochemicals	MEPA fruit pulp	MEPA seed
Alkaloids	+	+
Flavonoids	+	–
Steroids	–	–
Terpenoids	+	–
Glycosides	+	+
Saponins	–	+
Tannins	–	–
Phenols	+	+
Proteins	+	+
Carbohydrates	+	–

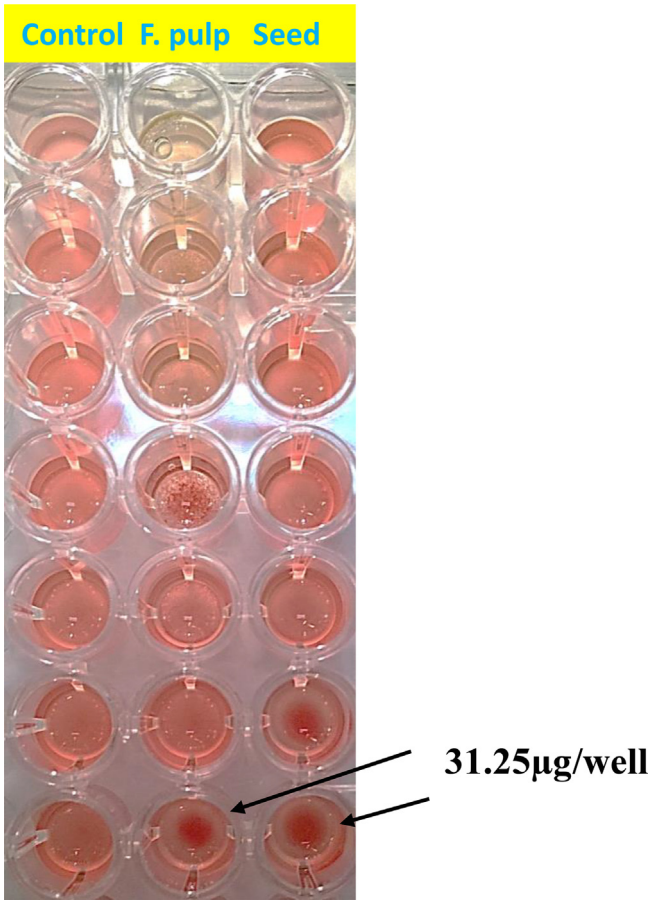


Fig. 4. Hemagglutination activity of MEPA fruit pulp and seed where both extract showed agglutination activity against RBCs at the concentration of 31.25 µg/well. Here, control well did not show any agglutination activity.

was 9.888 µg/ml. In case of IC_{50} value, the mean with different letters also express that they are significantly different at P < 0.05 level which is done by Duncan's multiple-range test. On the other hand, Nitric oxide (NO) is a crucial bio-regulatory molecule required for several physiological processes. Positive NO test also demonstrates that MEPA fruit pulp has higher inhibitory activity than MEPA seed in a dose-dependent manner but less inhibitory effect is found in both cases when compared with standard Ascorbic acid. Based on these results, it can be easily mentioned that *P. acidus* fruit pulp is a potential source of antioxidant compounds (Chakraborty et al., 2012).

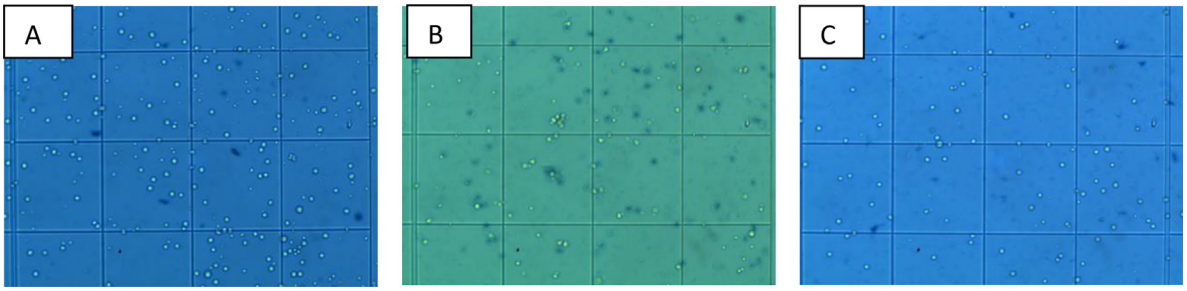


Fig. 5. Haemocytometric observation of EAC cells where **(A)** indicates viable EAC cells in control group without any treatment, **(B)** and **(C)** indicate MEPA fruit pulp and seed extract treated viable EAC cells respectively.

Table 2
Effects of MEPA fruit pulp and seed extract treated EAC cell growth inhibition in *in vivo* condition. Data are expressed as mean±SEM (n = 3).

Name of the Experiment	Dose (mg/kg body weight / day) (i. p.)	Viable EAC cells on day seven after inoculation of tumour (cells/ml)	% of cell growth inhibition
1. EAC + Control	No treatment	$(7.0\pm0.117)\times10^6$	———————
2. EAC + MEPA fruit pulp extract	100	$(2.4\pm0.019)\times10^6$	65.71%
3. EAC + MEPA seed extract	100	$(5.0\pm0.045)\times10^6$	28.57%

Again, cytotoxicity was determined following Brine Shrimp lethality bioassay at different concentrations to predict the toxic effect on normal cells (Saha et al., 2013). The MEPA fruit pulp and seed were exhibited lower toxicity against Brine Shrimp nauplii although the mortality rate of Brine Shrimp nauplii was increased by increasing the concentration of test samples. For MEPA fruit and seed, LC_{50} value was obtained as 485.005 μg/ml and 665.393 μg/ml repectively which would be considered as a potential cytotoxic and toxic substance as LC_{50} is <1 000 μg/mL (Islam et al., 2013). Hence, a comparison of LC_{50} value indicates that both experimental samples have lower toxic effects on Brine shrimp nauplii and their effect is statistically significant ($P<0.05$ by *t*-test). Based on the result of LC_{50} value, MEPA fruit pulp and seed can be considered as a promising biological agent for conducting further experimentations in future as they have low toxicity on normal cells (Islam et al., 2018a,b). Based on phytochemical analysis report, it can be further assessed that the desired plant samples specially MEPA fruit pulp might be a novel source of medicines than MEPA seed due to the possessing of effective phytocompounds those have great medicinal value (Bhandari et al., 2017).

Moreover, it is well established that lectins are carbohydrate-binding proteins and capable to agglutinate blood erythrocytes in *in-vitro* conditions (Kabir et al., 2013). Therefore, it can be hypothesized from the obtained result of hemagglutination activity test that the agglutination of mouse erythrocyte induced by MEPA fruit pulp and seed is possibly due to the presence of lectin or lectin-like protein. Further, the agglutination activity of MEPA fruit pulp at different wells compare to MEPA seed typically indicates that it is a good source of lectin or lectin-like carbohydrate-binding proteins. Hence, agglutination may occur when lectin interacts with sugar moieties present on the cell surface of mouse blood erythrocytes (Sultan et al., 2009).

EAC cell is commonly used for anti-proliferative study in worldwide. Besides, several studies have been already reported that plant-derived extracts have toxicity against EAC cells and possess anticancer activity in tumor-forming experimental animals (Kakoti et al., 2015). Mice in each group were sacrificed on day seven and then total intraperitoneal EAC cells were harvested to dilute into 1% normal saline. Viable cells were first identified by using trypan blue dye and counted by a hemocytometer following the method of (Tennant, 1964). Besides, the total number of viable cells in the treated groups was compared with those of the control group. Based on the result of cell count, viable EAC cells were found as the decreased number in case of MEPA fruit pulp and seed extract treated mice mentioned as $2.4x10^6$cells/ml and $5.0x10^6$-cells/ml compared to control mice that showed $7.0x10^6$cells/ml. However, it typically suggests the cell growth inhibition property mediated by MEPA fruit pulp and seed extract (Bala et al., 2010; Senthilkumar et al., 2008). Furthermore, more effective cell growth inhibition has been observed in MEPA fruit pulp treated group (65.71%) in compare to seed extract group (28.57%) based on the result. Therefore, it can be easily concluded that our experimental samples have the efficacy of anti-proliferative property against EAC cells where many other similar studies also support this activity (Al-Mamun et al., 2016b; Islam et al., 2014).

DAPI is a fluorescent stain that is extensively used in fluorescence microscopy and it can pass only through an intact cell membrane. Therefore, it is used to stain both live and fixed cells. Though it passes through the membrane of live cells but the effectiveness of the stain is lower (Kerr et al., 1972). Apoptotic morphological alterations such as fragmented nuclei and nuclear condensation

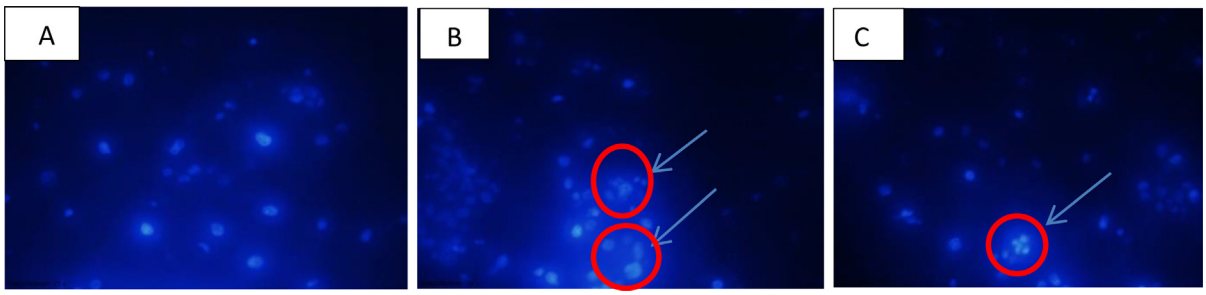


Fig. 6. The morphological changes of EAC cells under the fluorescence microscope induced by MEPA fruit pulp and seed extract. Here, **(A)** represents EAC cells derived from control mice showed regular and round-shaped nuclei while **(B)** and **(C)** indicates the EAC cells of MEPA fruit pulp and seed extract-treated mice with the appearance of morphological and structural changes of nuclei.

among the neighboring cells have been observed by fluorescence microscopy in treated groups compared to the control group (Al-Mamun et al., 2016a). Besides, more condensed and fragmented nuclei have been found in case of MEPA fruit pulp treated EAC cell images rather than the seed extract which may be due to the initiation of EAC cell apoptosis (Hasan et al., 2019; Zahan et al., 2019). These results indirectly indicate that lectin-like proteins present in *Phyllanthus acidus* fruit pulp and seed extract may be responsible for the induction of morphological alterations of EAC cells (Islam et al., 2014; Singh et al., 2017). Finally, it can be suggested that *P. acidus* fruit pulp has potential EAC cell growth inhibition activity because of its good antioxidant property along with other biological nature, and that's why it can be used as a novel anticancer drug development process after further molecular analysis.

5. Conclusion

The present study shows that there is strong biological evidence for *P. acidus* (L.) fruit pulp and seeds. Based on the above findings, MEPA fruit pulp directs high antioxidant activity, a considerable amount of essential phytochemicals, a low toxic impact on normal cells, and a good source of lectin protein compared to seeds. Besides, effective EAC cell growth inhibition properties with some remarkable morphological changes in the nucleus have also been identified in both cases, but a more effective result has been achieved in case of MEPA fruit pulp. To get relief from debilitating disorders, MEPA fruit pulp is, therefore, to be considered a more promising therapeutic source than seed. Further studies may be conducted in the future to identify specific bioactive molecules as a novel therapeutic agent for the treatment of cancer, diabetes, cardiovascular disorders, etc.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

All relevant data are within the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.01.004.

References

Al-Mamun, M.A., Akter, Z., Uddin, M.J., Ferdaus, K.M.K.B., Hoque, K.M.F., Ferdousi, Z., Reza, M.A., 2016a. Characterization and evaluation of antibacterial and antiproliferative activities of crude protein extracts isolated from the seed of Ricinus communis in Bangladesh. BMC Complement. Altern. Med. 16. https://doi.org/10.1186/s12906-016-1185-y.

Al-Mamun, M.A., Husna, J., Khatun, M., Hasan, R., Kamruzzaman, M., Hoque, K.M.F., Reza, M.A., Ferdousi, Z., 2016b. Assessment of antioxidant, anticancer and antimicrobial activity of two vegetable species of Amaranthus in Bangladesh. BMC Complement. Altern. Med. 16. https://doi.org/10.1186/s12906-016-1130-0.

Alam, A.K., Hossain, A.S., Khan, M.A., Kabir, S.R., Reza, M.A., Rahman, M.M., Islam, M. S., Rahman, M.A.A., Rashid, M., Sadik, M.G., 2016. The Antioxidative Fraction of White Mulberry Induces Apoptosis through Regulation of p53 and NFκB in EAC Cells. PLoS One 11,. https://doi.org/10.1371/journal.pone.0167536.t002 e0167536.

Andrianto, D., Widianti, W., Bintang, M., 2017. Antioxidant and Cytotoxic Activity of Phyllanthus acidus Fruit Extracts. IOP Conf. Ser. Earth Environ. Sci. 58. https://doi.org/10.1088/1755-1315/58/1/012022.

Bala, A., Kar, B., Haldar, P.K., Mazumder, U.K., Bera, S., 2010. Evaluation of anticancer activity of Cleome gynandra on Ehrlich's Ascites Carcinoma treated mice. J. Ethnopharmacol. 129, 131–134. https://doi.org/10.1016/j.jep.2010.03.010.

Banik, G., Bawari, M., Choudhury, M.D., Choudhury, S., Sharma, G.D., 2010. Some Anti-Diabetic Plants of Southern Assam. Assam Univ. J. Sci. Technol. 5, 114–119.

Bhandari, J., Muhammad, B.T., Thapa, P., Shrestha, B.G., 2017. Study of phytochemical, anti-microbial, anti-oxidant, and anti-cancer properties of Allium wallichii. BMC Complement. Altern. Med. 17. https://doi.org/10.1186/s12906-017-1622-6.

Brooks, R., Goldson-Barnaby, A., Bailey, D., 2020. Nutritional and Medicinal Properties of Phyllanthus Acidus L. (Jimbilin). Int. J. Fruit Sci. https://doi.org/10.1080/15538362.2020.1830013.

Chakraborty, R., Biplab, D., Devanna, N., Sen, S., 2012. Antiinflammatory, antinociceptive and antioxidant activities of Phyllanthus acidus L. extracts. Asian Pac. J Trop. Biomed. 2. https://doi.org/10.1016/S2221-1691(12)60343-8.

Dakah, A., Zaid, S., Suleiman, M., Abbas, S., Wink, M., 2014. In vitro propagation of the medicinal plant Ziziphora tenuior L. and evaluation of its antioxidant activity. Saudi J. Biol. Sci. 21, 317–323. https://doi.org/10.1016/j.sjbs.2013.12.002.

Devi, S.S., Paul, S.B., 2011. An Overview on Cicca acida (Phyllanthus acidus). Assam Univ. J. Sci. Technol. 7, 156–160.

Dubey, N.K., Kumar, R., Tripathi, P., 2004. Global promotion of herbal medicine: India's opportunity. Curr. Sci. 86, 37–41.

Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D., Guo, Z., 1985. Medicinal plants in therapy. Bull. World Health Organ. 63, 965–981. https://doi.org/10.1016/0378-8741(87)90016-x.

Figueroa, L.A., Navarro, L.B., Vera, M.P., Petricevich, V.L., 2014. Antioxidant activity, total phenolic and flavonoid contents, and cytotoxicity evaluation of Bougainvillea xbuttiana. Int. J. Pharm. Pharm. Sci. 6, 497–502.

Habib, R., Rahman, M., Mannan, A., Zulfiker, A.H., Uddin, M.E., Sayeed, M.A., 2011. Evaluation of antioxidant, cytotoxic, antibacterial potential and phytochemical screening of chlorofrom extract of Phyllanthus acidus. Int. J. Appl. Biol. Pharm. Technol. 2, 420–427.

Halliwell, B., 2006. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiol. 141, 312–322. https://doi.org/10.1104/pp.106.077073.

Hasan, M.M., Islam, M.S., Hoque, K.M.F., Haque, A., Abu Reza, M., 2019. Effect of Citrus macroptera fruit pulp juice on alteration of caspase pathway rendering anti-proliferative activity against Ehrlich's ascites carcinoma in Mice. Toxicol. Res. 35, 271–277. https://doi.org/10.5487/TR.2019.35.3.271.

Islam, F., Khanam, J.A., Khatun, M., Zuberi, N., Khatun, L., Kabir, S.R., Reza, M.A., Ali, M., Rabbi, M.A., Gopalan, V., Lam, A.K.Y., 2015. A p-menth-1-ene-4,7-diol (EC-1) from eucalyptus camaldulensis dhnh. triggers apoptosis and cell cycle changes in ehrlich ascites carcinoma cells. Phyther. Res. 29, 573–581. https://doi.org/10.1002/ptr.5288.

Islam, F., Khatun, H., Khatun, M., Ali, S.M.M., Khanam, J.A., 2014. Growth inhibition and apoptosis of Ehrlich ascites carcinoma cells by the methanol extract of Eucalyptus camaldulensis. Pharm. Biol. 52, 281–290. https://doi.org/10.3109/13880209.2013.834365.

Islam, M.S., Rahi, M.S., Jahangir, C.A., Rahman, M.H., Jerin, I., Amin, R., Hoque, K.M.F., Reza, M.A., 2018a. In Vivo Anticancer Activity of Basella alba Leaf and Seed Extracts against Ehrlich's Ascites Carcinoma (EAC) Cell Line. Evidence-based Complement. Altern. Med. 2018. https://doi.org/10.1155/2018/1537896.

Islam, S., Rahi, S., Koli, H.K., Jerin, I., Sajib, S.A., Hoque, K.F., Reza, A., 2018b. Evaluation of phytochemical, antioxidant, cytotoxicity and in vitro antibacterial activity of aqueous extract of Ganoderma lucidum cultivated in Bangladeshi habitat. Malaya J. Biosci. 5, 1–13.

Islam, S.M.A., Ahmed, K.T., Manik, M.K., Wahid, M.A., Kamal, C.S.I., 2013. A comparative study of the antioxidant, antimicrobial, cytotoxic and thrombolytic potential of the fruits and leaves of Spondias dulcis. Asian Pac. J. Trop. Biomed. 3, 682–691. https://doi.org/10.1016/S2221-1691(13)60139-2.

Jain, N.K., Singhai, A.K., 2011. Protective effects of Phyllanthus acidus (L.) Skeels leaf extracts on acetaminophen and thioacetamide induced hepatic injuries in Wistar rats. Asian Pac. J. Trop. Med. 4, 470–474. https://doi.org/10.1016/S1995-7645(11)60128-4.

Kabir, S.R., Nabi, M.M., Haque, A., Zaman, R.U., Mahmud, Z.H., Reza, M.A., 2013. Pea lectin inhibits growth of Ehrlich ascites carcinoma cells by inducing apoptosis and G2/M cell cycle arrest in vivo in mice. Phytomedicine 20, 1288–1296. https://doi.org/10.1016/j.phymed.2013.06.010.

Kabir, S.R., Nabi, M.M., Nurujjaman, M., Reza, M.A., Alam, A.H.M.K., Zaman, R.U., Khalid-Bin-Ferdaus, K.M., Amin, R., Khan, M.M.H., Hossain, M.A., Uddin, M.S., Mahmud, Z.H., 2015. Momordica charantia Seed Lectin: Toxicity, Bacterial Agglutination and Antitumor Properties. Appl. Biochem. Biotechnol. 175, 2616–2628. https://doi.org/10.1007/s12010-014-1449-2.

Kakoti, B.B., Hernandez-Ontiveros, D.G., Kataki, M.S., Shah, K., Pathak, Y., Panguluri, S.K., 2015. Resveratrol and Omega-3 Fatty Acid: Its Implications in Cardiovascular Diseases. Front. Cardiovasc. Med. 2. https://doi.org/10.3389/fcvm.2015.00038.

Kerr, J.F.R., Wyllie, A.H., Currie, A.R., 1972. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer 26, 239–257. https://doi.org/10.1038/bjc.1972.33.

Kim, H.J., Park, C.G., Varghese, R., Lee, J.Y., Kim, Y.O., Sung, G.H., 2017. In-vitro antioxidative, antiinflammatory properties of Aurea helianthus leaf extract a Korean traditional medicinal plant. Saudi J. Biol. Sci. 24, 1943–1947. https://doi.org/10.1016/j.sjbs.2017.11.046.

Kirtikar, K.R., Basu, B.D., 1987. Indian Medicinal Plants. International Book Distributors, Dehradun. Vol, I-IV.

Kumarappan, C.T., Thilagam, E., Mandal, S.C., 2012. Antioxidant activity of polyphenolic extracts of Ichnocarpus frutescens. Saudi J. Biol. Sci. 19, 349–355. https://doi.org/10.1016/j.sjbs.2012.04.004.

Lee, B.B., Cha, M.R., Kim, S.Y., Park, E., Park, H.R., Lee, S.C., 2007. Antioxidative and anticancer activity of extracts of cherry (Prunus serrulata var. spontanea) blossoms. Plant Foods Hum. Nutr. 62, 79–84. https://doi.org/10.1007/s11130-007-0045-9.

Marcocci, L., Maguire, J.J., Droy-Lefaix, M.T., Packer, L., 1994. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. Biochem. Biophys. Res. Commun. 201, 748–755. https://doi.org/10.1006/bbrc.1994.1764.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., McLaughlin, J.L., 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med. 45, 31–34. https://doi.org/10.1055/s-2007-971236.

Miah, M., Shimu, A.S., Mahmud, S., Omar, F.B., Khatun, R., Mohanto, S.C., Hoque, K.M. F., Reza, M.A., 2020. Methanolic Bark Extract of Abroma augusta (L.) Induces Apoptosis in EAC Cells through Altered Expression of Apoptosis Regulatory Genes. Evidence-based Complement. Altern. Med. 2020. https://doi.org/10.1155/2020/9145626

Moniruzzaman, M., Asaduzzaman, M., Hossain, M.S., Sarker, J., Rahman, S.M.A., Rashid, M., Rahman, M.M., 2015. In vitro antioxidant and cholinesterase inhibitory activities of methanolic fruit extract of Phyllanthus acidus. BMC Complement. Altern. Med. 15. https://doi.org/10.1186/s12906-015-0930-y.

Newman, D.J., Cragg, G.M., 2016. Natural Products as Sources of New Drugs from 1981 to 2014. J. Nat. Prod. 79, 629–661. https://doi.org/10.1021/acs.jnatprod.5b01055.

Nisar, M., Ali, S., Qaisar, M., 2011. Preliminary Phytochemical Screening of Flowers, Leaves, Bark, Stem and Roots of Rhododendron arboreum. Middle-East J. Sci. Res. 10, 472–476.

Olayaki, L.A., Irekpita, J.E., Yakubu, M.T., Ojo, O.O., 2015. Methanolic extract of Moringa oleifera leaves improves glucose tolerance, glycogen synthesis and lipid metabolism in alloxan-induced diabetic rats. J. Basic Clin. Physiol. Pharmacol. 26, 585–593. https://doi.org/10.1515/jbcpp-2014-0129.

Parejo, I., Codina, C., Petrakis, C., Kefalas, P., 2000. Evaluation of scavenging activity assessed by Co(II)/EDTA-induced luminol chemiluminescence and DPPH· (2,2-diphenyl-1-picrylhydrazyl) free radical assay. J. Pharmacol. Toxicol. Methods 44, 507–512. https://doi.org/10.1016/S1056-8719(01)00110-1.

Pasha, M.K., Uddin, S.B., 2019. Minor edible fruits of Bangladesh. Bangladesh J. Plant Taxon. 26, 299–313. https://doi.org/10.3329/bjpt.v26i2.44596.

Rajkapoor, B., Sankari, M., Sumithra, M., Anbu, J., Harikrishnan, N., Gobinath, M., Suba, V., Balaji, R., 2007. Antitumor and cytotoxic effects of Phyllanthus polyphyllus on ehrlich ascites carcinoma and human cancer cell lines. Biosci. Biotechnol. Biochem. 71, 2177–2183. https://doi.org/10.1271/bbb.70149.

Saeed, A., Sultana, B., Anwar, F., Mushtaq, M., Alkharfy, K.M., Gilani, A.H., 2014. Antioxidant and antimutagenic potential of seeds and pods of green cardamom (Elettaria cardamomum). Int. J. Pharmacol. 10, 461–469. https://doi.org/10.3923/ijp.2014.461.469.

Saha, A.K., Rahman, R., Shahriar, M., Saha, S.K., Azad, N.A., 2013. Screening of six Ayurvedic medicinal plant extracts for antioxidant and cytotoxic activity. J. Pharmacogn. Phytochem. 2, 181–188.

Senguttuvan, J., Paulsamy, S., Karthika, K., 2014. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for in vitro antioxidant activities. Asian Pac. J. Trop. Biomed. 4, S359–S367 https://doi.org/10.12980/APJTB.4.2014C1030.

Senthilkumar, N., Badami, S., Dongre, S.H., Bhojraj, S., 2008. Antioxidant and hepatoprotective activity of the methanol extract of Careya arborea bark in Ehrlich ascites carcinoma-bearing mice. J. Nat. Med. 62, 336–339. https://doi.org/10.1007/s11418-008-0237-0.

Siddhuraju, P., Becker, K., 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. J. Agric. Food Chem. 51, 2144–2155.

Siddhuraju, P., Manian, S., 2007. The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram (Macrotyloma uniflorum (Lam.) Verdc.) seeds. Food Chem. 105, 950–958. https://doi.org/10.1016/j.foodchem.2007.04.040.

Singh, V.K., Arora, D., Satija, N.K., Khare, P., Roy, S.K., Sharma, P.K., 2017. Intricatinol synergistically enhances the anticancerous activity of cisplatin in human A549 cells via p38 MAPK/p53 signalling. Apoptosis 22, 1273–1286. https://doi.org/10.1007/s10495-017-1404-0.

Stankovic, M.S., Curcic, M.G., Zizic, J.B., Topuzovic, M.D., Solujic, S.R., Markovic, S.D., 2011. Teucrium plant species as natural sources of novel anticancer compounds: Antiproliferative, proapoptotic and antioxidant properties. Int. J. Mol. Sci. 12, 4190–4205. https://doi.org/10.3390/ijms12074190.

Sultan, N.A.M., Kavitha, M., Swamy, M.J., 2009. Purification and physicochemical characterization of two galactose-specific isolectins from the seeds of trichosanthes cordata. IUBMB Life 61, 457–469. https://doi.org/10.1002/iub.174.

Tan, S.P., Tan, E.N.Y., Lim, Q.Y., Nafiah, M.A., 2020. Phyllanthus acidus (L.) Skeels: A review of its traditional uses, phytochemistry, and pharmacological properties. J. Ethnopharmacol. 253. https://doi.org/10.1016/j.jep.2020.112610.

Tennant, J.R., 1964. Evaluation of the trypan blue technique for determination of cell viability. Transplantation 2, 685–694. https://doi.org/10.1097/00007890-196411000-00001.

Uddin, G., Rauf, A., Arfan, M., Ali, M., Qaisar, M., Saadiq, M., Atif, M., 2012. Preliminary Phytochemical Screening and Antioxidant Activity of Bergenia caliata. Middle-East J. Sci. Res. 11, 1140–1142.

Unander, D.W., Webster, G.L., Blumberg, B.S., 1990. Records of usage or assays in Phyllanthus (Euphorbiaceae) I. Subgenera Isocladus, Kirganelia. Cicca and Emblica. J. Ethnopharmacol. 30, 233–264. https://doi.org/10.1016/0378-8741(90)90105-3.

Vuorela, P., Leinonen, M., Saikku, P., Tammela, P., Rauha, J.-, Wennberg, T., Vuorela, H., 2012. Natural Products in the Process of Finding New Drug Candidates. Curr. Med. Chem. 11, 1375–1389. https://doi.org/10.2174/0929867043365116

Yıldırım, A., Mavi, A., Oktay, M., Kara, A.A., Algur, Ö.F., Bilaloğlu, V., 2000. Comparison of antioxidant and antimicrobial activities of Tilia (Tilia argentea Desf ex DC), sage (Salvia triloba L.), and Black tea (Camellia sinensis) extracts. J. Agric. Food Chem. 48, 5030–5034.

Zahan, R., Rahi, M.S., Sheikh, M.C., Miyatake, R., Zangrando, E., Naz, T., Islam, M.A.A. A.A., Reza, M.A., 2019. Design, synthesis and X-ray structural studies of novel [acetonitrile-benzyl-3-N-(2, 4 dihydroxyphenylmethylene) hydrazinecarbodithioato-κ3-N′, S, O] nickel(II) complex that potently inhibit cell proliferation through regulation of apoptosis related genes. Appl. Organomet. Chem. 33. https://doi.org/10.1002/aoc.4601.

Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64, 555–559. https://doi.org/10.1016/S0308-8146(98)00102-2.