Abstract: This research study accomplished to explore the antioxidant activity as well as cytotoxicity assessment like brine shrimp lethality bioassay of distinct fractions of Randia dumetorum stem extract. To fractionate through soxhletion using sequential extraction techniques where the plant stem used as a powdered form with some specific solvents treatment. Here, we used to evaluate antioxidant activity, total antioxidant capacity determination, reducing power assay, ferric ions reduction using a simple method named ortho-phenanthroline color method, determination of total content of phenol and total flavonoids contents by aluminium trichloride method. In these studied we incorporate Ascorbic and Gallic acid as an antioxidant compound. It observed that, the content of proximate analysis moisture is 10.3%, total ash value is 4.76%, acid insoluble ash is 4.30% and water soluble ash value is 3.21%. For phytochemical screening, different extract of those solvents were utilized that disclosed the existence of alkaloids, reducing sugar, flavonoids, saponin, phenols, Tannic acid, amino acid and protein. In the case of brine shrimp lethality bioassay, methanol extract of stem effect to brine shrimp nauplii and exhibiting highest toxicity having LC₅₀ value 1.32 μg/ml as compared to standard dimethyl sulfoxide (LC₅₀ 1.31 µg/ml). These evaluations suggest that Randia dumetorum stems indicated a better source of antioxidants and hold important cytotoxic effect.

Keywords: Randia dumetorum, antioxidant, DMSO, cytotoxicity.
1. Introduction

Human body gradually faces various diseases from early to the old stage of life because of the oxidation reaction. In our body unstable free radicals gradually created because of natural biological and chemical process which is also called oxidative stress is the main culprit of cell damage. This cell damage is directly related to decay, diseases and death of human civilization [1]. From the dawn of civilization human are interrelated with various disease, decay and death. When they want to cure from different kind of illness then they start using plants as a medicine, as a consequence, they get outstanding therapeutic tools against diseases [2, 3]. Free radicals which are dangerous products created during normal biological process in our body. If the free radicals or reactive oxygen molecules is gradually created in our body which are detrimental for human cell is the initial responsible for various diseases and human life comes to an end in a moment of time. The antioxidant is a reducing agent suppresses the human cell damage induced by reactive oxygen species and try to minimize the oxidative stress in human body [4]. It is important factor to balance between reactive oxygen species and the inherent antioxidant potential activity of the body for maintaining a good health. During the attack of diseases, the use of medicinal supplements which are prepared from various medicinal plants certainly is used to balance the condition between free radicals and inherent antioxidant potential and improve the protective cellular defence system for leading a long period of human life in a better way. Now many of the pharmacists, doctors and researchers or scientists have attention an appreciation of antioxidants extracted from various alternative plants for the contribution to the indigenous systems of the world for the maintenance of the population health [5].

*Randia dumetorum* Lam. is a plant of medicinal important mostly neotropical genus of thorny shrubs or small trees grows up to 5 meters of height belongs to the family Rubiaceae popularly known as emetic nut [6]. Ripe fruits retain glycosides, essential oil also resin acid. Bark retain two coumarin glycosides, mannitol and saponins. Seed retain Pb (lead). Six saponins retain oleanolic acid as a glycone they identified as dumetoronins A, B, C, D, E and F [7]. In the case of Ayurveda, different parts of *Randia dumetorum* are employed to mollify vitiated pitta and kapha/mucous as well as to cure from skin diseases, cough, asthma, flatulence, ulcers, colic etc. The fruit of plant give rise to anti-inflammatory, emetic/inducing vomiting and abortifacient properties. To relieve pain of bruises and rheumatism tree bark is used externally [8]. Leaf parts of this plant was also investigated in previous study [5]. Till now, the antioxidant potential, proximate analysis and
cytotoxicity of various parts from *Randia dumetorum* stem did not analysis. Therefore, this research work done to analyze the antioxidant potentiality, proximate analysis and cytotoxicity of various fractions of stem of *Randia dumetorum*.

2. Materials

2.1 Instruments, equipments, reagents and chemicals

Laboratory glassware i.e. test tube, conical flask, measuring cylinder, volumetric flask, beaker, funnel, pipette, digital balance machine (AGN 220C, AXIS, Poland), soxhlet extractor, Oven dryer (UM 400, Memmert GmbH, Germany). A pH (PHS 25, Clida Instrument), Rotary evaporator etc. Besides, Folin-Ciocaltu reagent, Potassium mercuric iodide reagent, Wagner reagent, Picric acid solution reagent and Bismuth nitrate reagent, lead acetate solution, Benedict reagent, Fehling reagent, Ferric chloride, Potassium dichromate, Nitric acid, Ortho-phenanthroline, Potassium ferricyanide, Ammonium molybdate, Sodium potassium tertarate, Butylated Hydroxy Toluene (BHT), Sodium di-hydrogen orthophosphate, Ascorbic acid, Gallic acid, Dimethyl sulfoxide, Sulphuric acid, Trichloro acetic acid etc. All chemicals are purchased from Merk, Germany.

2.2 Plant Collection

The plant collected from Noakhali, Bangladesh on March 5, 2015 and identified by phytochemical based expert research group in which lab head was Md. Tanvir Hossain, Assistant professor, NSTU. Then the stems were separated from unnecessary or unexpected materials or plant parts. Then dried by Air under shade to avoid sunlight for about 4 weeks after cutting small pieces. The dried stems pieces further dried at oven for 24 hour at 40°C temperature. The sample was ground using high capacity grinder to form coarse powder. Resulting stems powder sample about 160 gm (yielding 20% w/w) kept in a vial in which there is no moisture and temperature is perfectly maintained until different investigation commenced.

3. Methods

3.1 Proximate Analysis

In food industry, proximate analysis is a reliable old techniques carry out to assess food quality for betterment of product and nutrition analysis as well as to evaluate purity and quality of herbal medications. According to standard protocols the proximate analysis, total ash value, the moisture content, water soluble ash value and acid insoluble ash value were determined in the samples [9].

3.1.1 Determination of Moisture Content
In a crucible precisely weighed 5 gm. of powdered of *Randia dumetorum* stems were taken. Then it was kept in an oven at 105–110 °C temperature. So that free moisture is eliminated easily. The moisture content (%) was evaluated by comparison between oven dried sample and Air dried sample.

3.1.2 Total Ash Value Determination

5 gm. of powdered *Randia dumetorum* stems were taken in a crucible of dried silica. Incineration is performed at 450 °C temperature, due to the elimination total amount of carbon. Cooled the sample. The total content of ash (%) was calculated by weighting compare to reference sample.

3.1.3 Acid Insoluble Ash Value Determination

Weight total ash and mixed with 2 N 25 ml hydrochloric acid and heated for 5 min. Then the solution was filtered and residue was taken of ash free filter paper and rinse carefully with warm water. Then the sample ignite at least 15 minutes at under 450 °C, cooled and weighted. The percentage of acid insoluble ash was evaluated compare to reference sample.

3.1.4 Determination of Water Soluble Ash Value

Weight total ash and mixed with 25 ml of water and boiled about 10 min. Then the solution was filtered and the insoluble residue was taken on filter paper. Then the sample ignite at least 15 minutes at under 450 °C, cooled and weighted. The weight difference denoted the content of water-soluble ash. The percentage of water soluble ash was evaluated compare to reference sample.

3.2 Extract Preparation

An effective and easy method is hot extraction in which soxhlet extractor is used where the probable sample material has a partial soluble in a solvent and the contamination is insoluble [10]. The material was exposed to parallel extraction with solvents in their increasing order of polarity. The parallel solvent extraction process was used for the preparation of different extracts of *R. dumetorum* stems. In this process, the material which is soluble in a solvent with the fixed range of polarity was extricated in the solvent and other residue further extricated with the next fixed solvent [11]. So, initially the parched stem powder of Randia dumetorum (about 160 g) were extricated with 600 ml n-hexane and after that the remaining dehydrated marc was extricated with chloroform, methanol and water by using soxhlet extractor for 8 hour at 58 to 80°C. In the case of water extract preparation, the parched marc was meshed for 3 days with distilled water and the residue was separated by filtration and the concentrated water extract was found. The various
solvents extracts were concentrated by the recovery of the solvent in a rotary evaporator. These concentrated extracts were then weighed and stocked for the assessments [5].

3.3 Preliminary Phytochemical Screening
The extract was put to initial phytochemical analysing. Phytochemical screening of minor proportion of newly found n-hexane, chloroform, methanol and water extricate from *R. dumetorum* stem sample were analysed to detect alkaloid, flavonoid, reducing sugar, saponin, phenolic compound, tannin, and amino acid using the standard methods [12].

3.4 Antioxidant Potential
Five well known and complex methods were applied to determine the antioxidant activity of the examined stem extract of *R. dumetorum* are total antioxidant capacity determination, reducing power assay, reduction of ferric ions by ortho–phenanthroline method, measurement of total phenol content and flavonoid content.

3.4.1 Total Antioxidant Capacity Determination
Phosphomolybdate method designed by Prieto was applied to evaluate the total antioxidant capacity of the plant stem extracts with slight modification [13]. 1 ml of reagent solution is prepared from 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate for this investigation and later this was mixed with an aliquot of 0.1 ml of sample solution. A capped tube in a water bath at 95°C for 90 min must be kept in carefully. After that the samples were cooled at normal room temperature and determined the absorbance at 695 nm against a blank sample. The total antioxidant activity was showed as the absorbance of the sample at 695 nm. It is recognized that high absorbance expressed the high antioxidant activity [5].

3.4.2 Reducing Power Assay
Reducing power assay is a popular method to measure the reduction potential of sample which are depends on the principle to convert ferric chloride to produce ferrous complex. Before the conversion firstly it was reacted with potassium ferricyanide to produce potassium ferrocyanide [9]. The presence of reducing agent for instance antioxidant substances in the crude extract causes the reduction of the ferricyanide (Fe3+) complex to the ferrous form (Fe2+) has an absorption maximum at 700 nm by the following Oyaizu method with a minor modification compared with standard Butylated Hydroxy Toluene (BHT) at various concentrations [14]. It is easily said that higher absorbance of the reaction mixture expressed the higher reducing potential [12].

3.4.3 Total Phenolic Content Determination
The phenolic concentration of plant’s extract of stem is determined by spectrophotometric method [15]. From a gallic acid calibration curve, the phenolic concentration of extracts was estimated. To prepare a calibration curve, 0.5 ml aliquots of concentration ranging from 500 μg/ml to 3.9 μg/ml gallic acid solutions were mixed with 2.5 ml (75 g/L) sodium carbonate and 2.5 ml Folin-Ciocalteu reagent (diluted ten times with water) which is a combination of phosphomolybdate and phosphotungstate reduced the polyphenols containing methanolic and water solution of the extract (concentration 1 mg/ml), thereby producing blue colored complex [5]. The sample was there after incubated at 45°C temperature at a in a thermostat for 45 min. The absorbance was measured using spectrophotometer at 765 nm against reagent blank. After plotting the absorbance in ordinate against the concentration in abscissa a linear relationship was obtained to determine the samples gallic acid equivalent from the absorbance detected which was expressed as mg of GAE per g of sample extract.

3.4.4 Total Flavonoid Content Determination
The flavonoids content was measured using quercetin as a reference compound by aluminium trichloride method in which a complex flavonoid-aluminium is found [16]. Each plant extracts (0.5 ml of 1:10 g/ml) in ethanol was separately blended with 1.5 ml of ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of water. It kept at room temperature for 30 min; the absorbance of the reaction mixture was assessed at 415 nm. At concentration 0, 0.25, 0.50, 0.75, 1.00 mg per ml in ethanol a calibration curve was plotted by making Quercetin solution. Total content of flavonoids was determined as mg of Quercetin equivalent per gram (mg per g of dry mass), which is used as a reference compound using an equation.

3.4.5 Reduction of Ferric Ions by Ortho – phenanthroline Color Method
The stem extract reduction of ferric ions was evaluated by o-phenanthroline method [9]. The mixture consists of 1 ml o-phenanthroline (5 mg in 10 ml methanol), 2 ml of 0.2 mM ferric chloride and 2 ml of various concentrations (31.25–500 μg/ml) of the extracts were incubated at ambient temperature for 10 min, and then the absorbance was measured at 510 nm with reference standard sample. Here ascorbic acid (AA) and gallic acid (GA) used as a reference sample.

3.5 Brine Shrimp Lethality Bioassay
The brine shrimp lethality be regarded as a simple and widely used bioassay guide for analyzing plant extract lethality for the isolation of antitumor and cytotoxic activity from medicinal plants.
The procedure of Meyer et al. [17] with some modification followed for this analysis, the crude methanolic lethality and aqueous stem extracts of *R. dumetorum* was measured because of phytochemical analysis. Firstly, *Artemia salina* was placed in 1 liter of sea water which is collected from pet shop, aerated for 48 hour at 37°C to hatch and after that nauplii was observed. In the present study, 100 μl of Dimethyl sulfoxide (DMSO) was only added as a negative control to each of three pre-marked glass containers containing 5 ml of simulated sea water and 10 shrimp nauplii to use as control groups. Ten living nauplii were added in each test tube. 4 mg of each sample including both aqueous and methanol extracts were taken in two separate vials and samples were thoroughly mixed in the DMSO. Each sample with marking for different concentrations taken at 10 test tubes. So 24 hours later, the containers were observed using a magnifying glass and the numbers of survivors were find out. At different concentrations, dissimilar mortality rates were found on each test samples; the 50% concentration mortality data of brine shrimp nauplii (median lethal concentration, LC₅₀) were analyzed by a curve of percentage of the shrimps killed versus the logarithm of the sample concentration.

4. Result and Discussion

4.1 Proximate Analysis

Proximate analysis of *R. dumetorum* reveals the content of moisture, total ash content, water soluble ash and acid insoluble ash were found 10.3%, 4.76%, 3.21% and 4.30%, respectively subjected in Figure 1.

![Proximate analysis of R. dumetorum stem](image)

Figure 1: Percentage of proximate analysis of *R. dumetorum* stem

From the Figure 1, it is clear that low percentage of moisture content of the stem of *R. dumetorum* reveals that it would slow down the growth of microorganism and long life storage property would be high. Moreover, knowing the moisture content of a substance helps to determine if that substance is fit for a specific use. The ash content of 4.76% indicates that high mineral element
existence in the stem besides it retain various inorganic radicals like carbonate, calcium silicate and phosphate etc. Total ash value determination is important because it indicates the crude medicinal quality and purity [9].

4.2 Phytochemical Analysis

Such type of secondary metabolites or bioactive compounds like alkaloid, flavonoid, reducing sugar, phenolic compounds, saponin, protein, tannin and amino acid etc. are vital for human health. So, phytochemical analysis of various stem extract of *Randia dumetorum* was experienced to observe bioactive compounds (Table 1)

Table 1. Qualitative Chemical Screening of Different Extracts of Randia dumetorum Stem

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Phyto-chemical composition</th>
<th>Outcomes of different extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n-hexane</td>
</tr>
<tr>
<td><em>Randia dumetorum</em></td>
<td>Alkaloid</td>
<td>–</td>
</tr>
<tr>
<td>(stem)</td>
<td>Reducing Sugar</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Saponin</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Phenolic compound</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Tannin</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Gum, Protein &amp; Amino acid</td>
<td>–</td>
</tr>
</tbody>
</table>

(+): Present, (-): Absent, (++): Significantly present

N-hexane contains only flavonoid, methanol contains all of the studied metabolites except alkaloid, saponin, protein and amino acid while chloroform contains only one and again water extract also contains four. The previous study of leaves extract contains flavonoid, decreasing sugar, phenolic compound, saponin, tanin and glycoside [5].
4.3 Antioxidant Activity

4.3.1 Total Antioxidant Activity

A standard curve of total anti-oxidant activity of the different extracts plotted in Fig. 2 the concentration versus absorbance at 695 nm based on the reduction of versus concentration of Mo (VI) to Mo (V). It determines how much electrons or radicals contributed by a given antioxidant molecule and determined the capacity of biological samples maintain standard conditions.

Figure 2: Curve of total anti-oxidant activity of *R. dumetorum* stem

The phosphomolybdate procedure is quantitative, since the total antioxidant capacity for various extract is denoted as ascorbic acid equivalents was exposed in Figure 3.

**Total Antioxidant Capacity (AAE/gm)**

Figure 3: Curve of total antioxidant capacity of various solvent extracts for 500 ppm at 695 nm

From Figure 3, the antioxidant activity of various solvent extract (500 µg/ml) of *R. dumetorum* stem existed at this order: water (38.3 AAE/g) < methanol (141.6 AAE/g) < Chloroform (340
AAE/g) < n-hexane (433.3 AAE/g). The current research demonstrated that n-hexane extract exposed the highest total antioxidant capacity for phosphomolybdate lessening.

**4.3.2 Reducing Power Assay**

This capability may work for a compound as an indicator in which the capability of the extract to contribute electron and convert Fe$^{3+}$ to Fe$^{2+}$ in a reaction. The reducing power ability of various extracts of *R. dumetorum* stem and BHT was used as a standard antioxidant. This comparison is displayed clearly in Figure 4. The absorbance at 510 nm for different extracts rises linearly with the growth in concentration which is shown in Figure 4.

![Graph showing reducing power assay](image)

Figure 4: Comparison of dissimilar stem extracts of *R. dumetorum* with standard (BHT)

Figure 4 elucidates that at 500 μg/ml concentration the n-hexane fraction reduced the Fe$^{3+}$ to Fe$^{2+}$ more effectively (1.872) as compared to the fractions of chloroform, methanol and water (1.548, 1.431 and 0.738), respectively.

**4.3.3 Determination Total Phenolic and Flavonoid Content**

Phenol is significant plant ingredients due to their oxygen molecule quencher hydroxyl groups. In the primary phytochemical screening, only methanol and water extracts exhibited the existence of phenol and so these two extracts were tested for total phenolic content in which total phenolic content of this stem extract was denoted as milligrams of gallic acid equivalents (GAE). The content of phenol at methanol and water extracts (32.9 GAE/g and 8.0 GAE/g) in which methanol extract showed higher concentration. The aluminium chloride colorimetric method is generally used to detect the complete flavonoid in which Al$^{3+}$ shows color that gives a abundant absorbance at 415 nm. In group test results the flavonoid was found in n-hexane and ethanol. The content of
flavonoid (22.0 & 27.7 mg quercetin equivalent/g of extract) was found in N-hexane and methanol stem extracts of *R. dumetorum* (Table 2).

Table 2. Total Phenolic and Flavonoid Content of *R. dumetorum* stem

<table>
<thead>
<tr>
<th>Name of Plant Extract</th>
<th>Total Phenolic content (mg gallic acid equivalent/gm)</th>
<th>Total flavonoid (mg quercetin/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-hexane</td>
<td>−</td>
<td>22.0</td>
</tr>
<tr>
<td>Chloroform</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Methanol</td>
<td>32.9</td>
<td>27.7</td>
</tr>
<tr>
<td>Water</td>
<td>8.0</td>
<td>−</td>
</tr>
</tbody>
</table>

**4.3.4 Reduction of Ferric Ions by Ortho – phenanthroline Color Method**

In this method, 1, 10-o-phenanthroline was used in which Fe$^{2+}$ reacts rapidly to forms a red colored complex. It has a strong absorption capacity in the visible spectrum at a wavelength of 510 nm and subsequently the conversion of Fe$^{3+}$ to Fe$^{2+}$ is obtained because of the reduction of this synthesized compounds. The degree of change in color indicates the reduction activity of the compounds. At 510 nm, the measure of ferric ions reducing potency have been used. In this method, Gallic acid & Ascorbic acid have been used as standard [9].

![Figure 5: Comparative study of dissimilar stem extracts with standard through ortho phenanthroline color method.](image-url)
Figure 5 displays the comparative study of various extracts of stem of *Randia dumetorum*. It is important to denote that the n-hexane extract and chloroform extract displayed noticeable antioxidant activity and the values (2.013 and 0.682) are significantly higher than the standard antioxidants (0.324 and 0.167) tested at 500 µg/ml. In addition, the methanol and water extracts (0.430 and 0.245) also give brilliant anti-oxidant activity at 500 µg/ml.

### 4.4 Lethality Bioassay

To explore the cytotoxicity of *R. dumetorum* stem brine shrimp lethality bioassay is performed. In this study, LC$_{50}$ values of crude methanol were found to be 1.32 µg/ml which is comparable with the negative control dimethyl sulfoxide revealed LC$_{50}$ at a concentration of 1.31 µg/ml (Table 3). Besides water extract showed least amount (1.065 µg/ml) of this property. So methanol crude extract showed magnificent antitumor and cytotoxicity activity than water extract. There were no mortality in the negative control groups because of the activity of the test agents.

Table 3: Effect of Extract of *R. dumetorum* Stem on Brine Shrimp Nauplii

<table>
<thead>
<tr>
<th>Sample/Extract</th>
<th>LC$_{50}$</th>
<th>Regression Equation</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl Sulfoxide (negative control)</td>
<td>1.31</td>
<td>$y = 34.024x + 5.0963$</td>
<td>0.9518</td>
</tr>
<tr>
<td>n-hexane</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Chloroform</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Methanol</td>
<td>1.32</td>
<td>$y = 27.92x + 12.993$</td>
<td>0.9735</td>
</tr>
<tr>
<td>Water</td>
<td>1.065</td>
<td>$y = 25.964x + 22.338$</td>
<td>0.9245</td>
</tr>
</tbody>
</table>

### 5. Conclusion

The stem of plant *Randia dumetorum* was investigated for its physicochemical, cytotoxicity, antioxidant and phytochemical screening. From the investigation it is easily said that the whole plant of *Randia dumetorum* is reported to exhibit good medicinal values in traditional system of medicines especially for various diseases. It is also denoted from the above conversation that these extracts showed an important anti oxidative stress potential and could suppress the continuous radical chain reaction and obtained good high storage life property and high quantity of mineral elements. Further study, however, is required for the proof to detect and analysed the active
chemical compounds liable for the other involved properties and also identify and proof of the better treatment of cancer or antitumor activity for its cytotoxicity properties [5].

Acknowledgment
The authors are indebted to the ACCE Department, NSTU, for giving the lab facilities for this research.

References


Dedication
Not mentioned.

Conflicts of Interest
There are no conflicts to declare.

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