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## ANTIOXIDANT AND CYTOTOXICITY POTENTIAL OF ALCOHOL AND PETROLEUM ETHER EXTRACT OF *BORASSUS FLABELLIFER* LINN.

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**ABSTRACT:** This study was subjected to investigate antioxidant and cytotoxic properties of ethanol and petroleum ether extracts of *Borassus flabellifer* Linn, expressed as BFA and BFP respectively. DPPH free radical scavenging method, total flavonoid, total phenol, total antioxidants and reducing power of the two extracts were assessed for antioxidant properties and cytotoxicity potentials were assessed by the brine shrimp lethality test. In DPPH test the lowest IC<sub>50</sub> value was 32.59 mg/ml for BFA and highest was 1388.46 mg/ml for BFP and compared with standard ascorbic acid. In cytotoxicity test LC<sub>50</sub> value was found 32.36 µg/ml for BFA and 616.59 µg/ml for BFP. So alcohol extract is more potent than petroleum ether extract. The total Flavonoid contents of the test samples were 3.57 ± 1.26 and 17.41±1.89 mg/g quercetin equivalents for BFA and BFP respectively. The total phenol contents of the test samples were 707.08 ± 8.84 and 28.75 ± 6.48 mg/g galic acid equivalents for BFA and BFP respectively and also in total antioxidant assay BFA contains 0.68 ± 0.17 and BFP contains 527.38 ± 3.93 mg/g ascorbic acid equivalents. *Borassus flabellifer* may possess powerful antioxidant as well as good cytotoxic potential.

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**INTRODUCTION:** Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centered free radicals and other reactive oxygen species (ROS), which are continuously, produced *in vivo*, result in cell death and tissue damage. The role of oxygen radicals have been implicated in several diseases, including cancer, diabetes, cardiovascular disease and aging <sup>1</sup>. Lipid peroxidation of fats and fatty acids in food not only results in their spoilage but is also a source of peroxy and hydroxyl radicals that are associated with carcinogenesis, mutagenesis and aging <sup>2,3</sup>.

Therefore antioxidants that scavenge these reactive oxygen species and free radicals are of major importance in preventing the onset and progression of many diseases caused by oxidative stress. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are very effective and are used for industrial processing, but they may possess side effects and toxic properties that affect human health <sup>4-6</sup>. The search for antioxidants from natural sources has received much attention and efforts have been put into the identification of compounds that can act as suitable antioxidants<sup>7</sup>.

Besides, phenolic compounds and flavonoids are also widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc<sup>8</sup>. As crude extracts of herbs and spices and other plant materials, rich in phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. While, flavonoids are a group of polyphenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action<sup>9</sup>.

*Borassus flabellifer* Linn of the Arecaceae family, locally called as Tal, English Name: Palmyra palm, is a tall tree attaining a height of about 30m, with a black stem and crown of leaves at the top; leaves are 0.9-1.5m in diameter, palmately fan shaped, petiole edges with hard horny spinescent serratures; flowers are unisexual, while male are spadix branched, female ar spadix simple; fruits are large, sub-globose drupes, on the greatly enlarged perianth. Trees can live upto more than 100 years. The rate of growth has been estimated at about 3 cm per year. This plant is widely distributed and cultivated in tropical Asian countries such as Thailand, Bangladesh, India, Myanmar, Sri Lanka, Malaysia, etc<sup>10-12</sup>.

*B. flabellifer* is used in folk medicine for multiple purposes, such as a stimulant, anti-laprotic, diuretic, antiphlogistic. The fruits are stomachic, sedative, laxative and aphrodisiac in nature useful in hyperdipsia, dyspepsia, flatulence, skin diseases, heamorrhages, fever and general debility. The roots and juice of the plant are useful in inflammatory reactions. The ash obtained by burning the inflorescence is a good antacid antiperiodic, and is useful in heart burn, spleenomegaly and in bilious fever<sup>13-14</sup>.

Studies on this plant have revealed the presence of several steroidal saponins<sup>10-12</sup>, a polysaccharide<sup>15</sup>, and a triterpenes<sup>16</sup>. The fresh pulp is reportedly rich in vitamins A and C<sup>17</sup> while the fresh sap is a good source of vitamin B-complex<sup>18</sup>. Male inflorescence constitutes spirostane-type steroid saponins like borassosides and dioscin. It also contains 20 known steroidal glycosides<sup>19</sup> and carbohydrates like sucrose<sup>20</sup>. *Borassus flabellifer* Linn. has been widely studied for its antidiabetic activity<sup>19, 21</sup>.

Flowers of *B. flabellifer* were investigated for analgesic and antipyretic effects<sup>22</sup>, anti-inflammatory activity, hematological and biochemical parameters<sup>23-24</sup>, immunosuppressant property<sup>16</sup>. Pellets of *B. flabellifer* Linn. showed a significantly reduced capacity to mount a delayed-type hypersensitivity (DTH)<sup>25</sup> and flour from the young shoots of the *B. flabellifer* tested for mutagenicity<sup>26</sup>, mitogenic activity<sup>27</sup>, neurotoxic effect<sup>28</sup>. Therefore, the present study evaluated the antioxidant and cytotoxic properties of the alcoholic (BFA) and petroleum ether (BFP) extract of *B. flabellifer* roots.

## MATERIALS AND METHODS:

### Collection and Preparation of the Plant material:

The root of *B. flabelifera* was collected in March, 2011 from Jahangirnagar University, Savar, Dhaka, Bangladesh. The sample was identified by experts in Bangladesh National Herbarium, Mirpur, and Dhaka. The accession numbers is 37896.

**Extraction of the Plant material:** The fresh root was washed with distilled water, minced into small pieces; the tested materials were sun dried for seven days. Extraction was performed at room temperature. About 200 g of dried, ground plant material were soaked in 98% ethanol and petroleum ether for 48 h, stirring a sterilized glass rod, filtered, and the solvent removed in vacuum using a rotary evaporator at a temperature below 40°C. Extracts were stored in sample glass ware in the refrigerator prior to use.

**Chemicals and drugs:** DPPH (1, 1-diphenyl, 2-picrylhydrazyl), TCA (trichloroacetic acid), Gallic acid and ferric chloride were obtained from Sigma Chemical Co. USA; ascorbic acid was from SD Fine Chem. Ltd. India, ammonium molybdate from Merck, Germany.

### Antioxidant Activity Test:

- Determination of Total Phenolic Content:** The total phenolic content of extracts was determined using Folin-Ciocalteu method<sup>29</sup>. The extracts were oxidized with Folin-Ciocalteu reagent and were neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 760 nm after 60 min. using gallic acid as standard total phenolic content was expressed as mg GA equivalent/gm of extract.

**2. Determination of Total Flavonoids Content:**

The flavonoids content was determined using a method as described by Kumaran and Karunakaran<sup>30</sup> using quercetin as a reference compound. 1 mg of plant extract in methanol was mixed with 1ml aluminium trichloride in Ethanol (20 mg/ml) and a drop of acetic acid, and then diluted with Ethanol to 25 ml. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 1 mg of plant extracts and a drop of acetic acid, and then diluted to 25 ml with ethanol. The absorption of standard quercetin solution (0.5 mg/ml) in methanol was measured under the same conditions.

**3. Determination of Total Antioxidant Capacity:**

The antioxidant activity of the extracts of *L. purpureus* were evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.*,<sup>31</sup>. The assay is based on the reduction of Mo (VI)-Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. 0.3 ml extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer (Shimadzu, UV-150-02) against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

**4. DPPH Radical Scavenging Activity:** The free radical scavenging capacity of the extracts was determined using DPPH<sup>32-33</sup>. A methanol DPPH solution (0.004% w/v) was mixed with serial dilutions (0 to 500 µg) of *L. purpureus* extracts and after 10 min; the absorbance was read at 515 nm using a spectrophotometer. Ascorbic acid was used as a standard. The inhibition curve was plotted and IC<sub>50</sub> values were calculated.

**5. Reducing Power:** The reducing power of *L. purpureus* extractives was determined according to the method previously described by Oyaizu<sup>34</sup>. Different concentrations of *L. purpureus* extracts in 1 ml of distilled water was mixed with

phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl<sub>3</sub> (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as a reference standard. Phosphate buffer (pH) was used as blank solution.

**Cytotoxic Activity:**

**1. Brine Shrimp Lethality Bioassay:** Cytotoxic activity of the plant extracts was determined by brine shrimp lethality bioassay method<sup>35</sup>. It is a recent development in the assay procedure of bioactive compounds, which indicates cytotoxicity as well as a wide range of pharmacological activities (e.g. anticancer, antiviral, insecticidal, pesticidal, AIDS, etc.) of the compounds<sup>36</sup>. The assay is considered as a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacterial toxins, pesticides and cytotoxicity testing of dental materials. The eggs of Brine Shrimp were hatched in a tank at a temperature around 37°C with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. Stock solution of the sample was prepared by dissolving required amount of extract in specific volume of pure dimethyl sulfoxide (DMSO). With the help of a pasteur pipette nauplii were exposed to different concentrations of the extracts.

**2. Preparation of Test Groups:** For the experiment, 20 mg of extract were dissolved in 1ml of DMSO and adjusted up to 20 ml by 3.8% NaCl. Then the solutions of varying concentrations (500, 200, 100, 50, 20, 10, 5, 1 µg/ml) were obtained by serial dilution technique. Each test tube contained about 5 ml of sea water with 10 shrimp nauplii.

**3. Counting of Nauplii:** The test tubes were kept at room temperature for about 24 hours and then, percent of mortality of nauplii was counted with the help of a magnifying glass.

The rate of mortality of nauplii was found to be increased in concentration of each of the samples. The median lethal concentration (LC<sub>50</sub>) was determined using the probit analysis method described by Finney<sup>37</sup>, as the measure of toxicity of the plant extract.

## RESULTS AND DISCUSSION:

**DPPH Free Radical Scavenging Assay:** The antioxidant activity of ethanol and petroleum ether extracts of root of *B. flabellifer* was determined by using the DPPH scavenging assay. The DPPH assay is often used to evaluate the ability of antioxidants to scavenge free radicals which are known to be a major factor in biological damage caused by oxidative stress. This assay is known to give reliable information concerning the antioxidant ability of the tested compounds<sup>38</sup>.

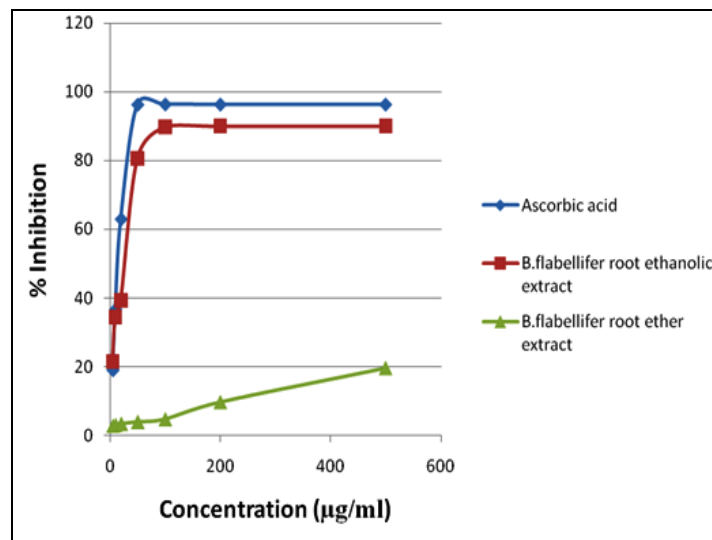
The principle of the assay is based on the color change of the DPPH solution from purple to yellow as the radical is quenched by the antioxidant<sup>39</sup>. The ethanol extract exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC<sub>50</sub>) at a concentration of 32.59 mg/ml (Figure 1). The corresponding IC<sub>50</sub> for petroleum ether extract was 1388.46 mg/ml.

**TABLE 1: TOTAL FLAVONOID AND TOTAL ANTIOXIDANT CONTENTS OF DIFFERENT EXTRACT OF *B. FLABELLIFER***

Sample	Total Flavonoid content (mg/g quercetin equivalents)	Total Phenol content (mg/g gallic acid equivalents)	Total Antioxidant capacity (mg/g ascorbic acid equivalents)
BFA	3.57 ± 1.26	707.08 ± 8.84	0.68 ± 0.17
BFP	17.41 ± 1.89	28.75 ± 6.48	527.38 ± 3.93

The Total Antioxidants capacity of different solvent extracts was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. The total antioxidants capacity of the test samples were calculated using the standard curve of Ascorbic acid ( $y=0.0063x + 0.0983$ ;  $R^2=0.9904$ ). The maximum total flavonoid (17.41±1.89 mg/g) was shown by petroleum ether extract of *B. flabellifer* L. and the minimum was shown by ethanol extract *B. flabellifer* (L.) (3.57 ± 1.26 mg/g). Total phenol content of the extract was evaluated by using Folin-Ciocalteu method and expressed as gallic acid equivalent. Alcohol extract of *B. flabellifer* showed higher phenolic content of 707.08 ± 8.84 mg/g and petroleum ether extract showed minimum total phenol content of 28.75 ± 6.48 mg/g.

Ascorbic acid which had been used as standard and had an IC<sub>50</sub> value of 18.90 mg/ml.



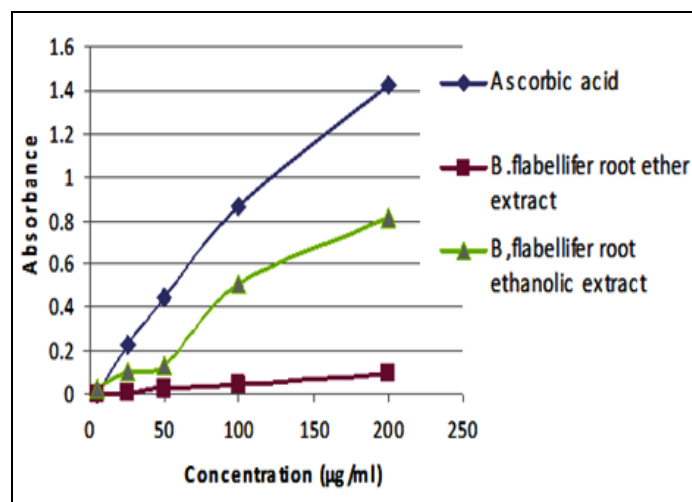
**FIGURE 1: COMPARATIVE DPPH RADICAL SCAVENGING ACTIVITY OF *B. FLABELLIFER* ROOT EXTRACTS AND ASCORBIC ACID**

**Total Flavonoid, Total Phenol and Total Antioxidant Activity:** The total flavonoid, total phenol and total antioxidant contents of *Borassus flabellifer* L. were expressed in quercetin, gallic acid and ascorbic acid equivalents respectively that are shown in **Table 1**.

Different studies suggest that different types of polyphenolic compounds such as flavonoids, phenolic acids which are found in plants have multiple biological effects, including antioxidant activity<sup>40</sup>. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1 g was daily ingested from a diet rich in fruits and vegetables<sup>41,42</sup>.

**Reducing Power:** By using the potassium Ferricyanide reduction method, the reductive capabilities of the plant extracts was identified in comparison with ascorbic acid which demonstrated at figure 2. The reducing power of the extracts was moderately strong while increasing dose it shows little increment.





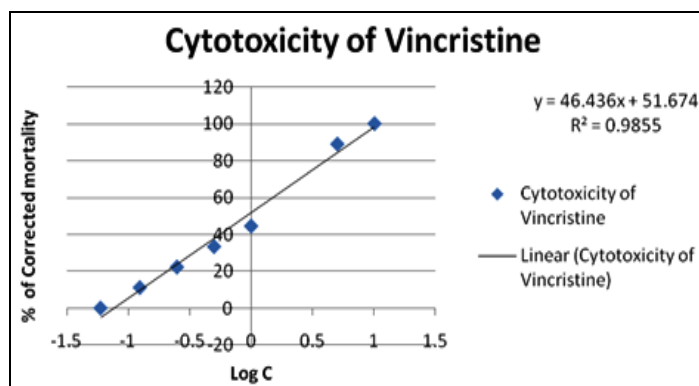
**FIGURE 2: REDUCING POWER OF THE ALCOHOL AND PETROLEUM ETHER EXTRACT OF *B. FLABELLIFER* AND ASCORBIC ACID**

**Cytotoxicity Screening:** In the present bioactivity study, the two crude extracts and pure compounds showed positive results indicating that the test samples are biologically active. The ethanol and petroleum ether extract of the dried bark of *B. flabellifer* were subjected to brine shrimp lethality bioassay following the procedure which has been utilized by Meyer *et al.*, 1982. The results of the different extracts of *B. flabellifer* (% mortality at different concentrations and  $LC_{50}$  values) were shown in **Table 2** and **Figure 3**.

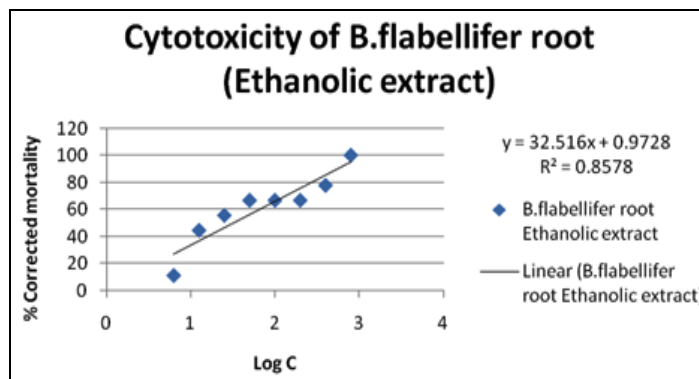
Vincristine sulphate (VS) was used as positive control and the  $LC_{50}$  was found as 0.92 µg/mL. Compared with the negative control, VS (positive control) gave significant mortality and the  $LC_{50}$  values of the different extractives were compared with negative control. Each of the test samples showed different mortality rates at different concentrations, the percentage mortality increased with an increase in concentration. The variation in results may be due to the difference in the amount and kind of cytotoxic substances (e.g. tannins, flavonoids, triterpenoids, or coumarins) present in the crude extracts. The  $LC_{50}$  values of ethanol and petroleum ether were found to be 32.36 µg/mL and 616.59 µg/mL respectively (**Table 2**).

**TABLE 2:  $LC_{50}$  VALUES OF THE TWO EXTRACTS OF *B. FLABELLIFER* AND STANDARD**

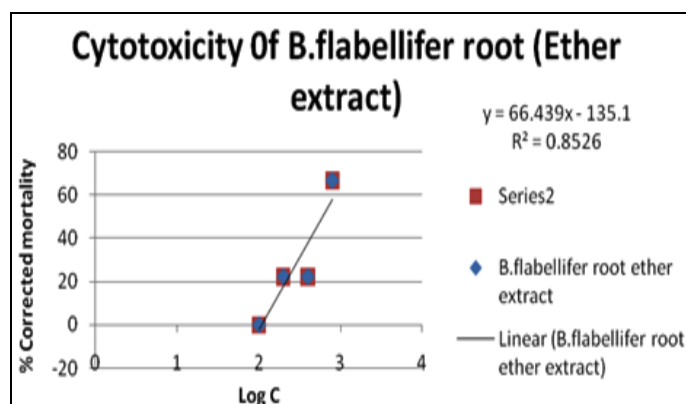
Test Samples	Regression Line	R2	$LC_{50}$ (µg/ml)
Vincristine	$y = 45.436x + 51.674$	0.985	0.92
BFA	$y = 32.516x + 38.9728$	0.858	32.36
BFP	$y = 66.439x - 135.1$	0.853	616.59



A



B



C

**FIGURE 3: CYTOTOXICITY STUDY**

A. Cytotoxicity of vincristine sulfate, B. Cytotoxicity of ethanol extract and C. Cytotoxicity of petroleum ether extract

**CONCLUSION:** In conclusion, it was observed from the present study that both ethanol and petroleum ether extract of *B. flabellifer* have significant natural antioxidants while ethanol extract of *B. flabellifer* has the potential cytotoxicity where the percentage mortality increased with an increase in concentration. But further pharmacological studies are required to be undertaken to understand the underlying possible mechanisms of the observed activities as well as need to isolate, purify, and characterize active phytochemicals responsible for these bioactivities.

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