Quercetin and Vincristine Potential of Methanolic Extract from Arjun Tree

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Abstract

Polyphenolic compounds like Gallic acid, ethyl gallate, quercetin, kempferol, proanthocyanidin, EPGA, resveratrol have been familiar as neuteraceuticals for possessing anti-oxidant, anti-inflammatory, and anti-cancer activities. Arjun Tree (Terminalia Arjuna) is a very ancient plant which contains many reported polyphenolic compounds having antifungal, cardioprotective, anti-mutagenic, anti-inflammatory, anti-allergic, anti-bacterial, cytotoxic, anti-oxidant properties. Here we reported methanolic extract of Terminalia arjuna, which neutralized 50% (IC50) of free radicals at a concentration of 3.29 μg/ml, while the standard neutralizes 50% (IC50) of free radical at a concentration of 1.92 μg/ml. The total phenolic content was 449.8 μg/ml of dry weight of extract, expressed as gallic acid equivalents. The total flavonoid concentration was 206.91μg/ml, expressed as quercetin equivalents. In the brine shrimp lethality test, LC50 obtained from the line slope were 0.419 and 12.5, which is greater than that of 0.781 μg/ml for standard vincristine sulphate, aqueous fraction.

Keywords: Polyphenolic Compounds, Methanolic Extract, Antioxidant, Cytotoxicity.

Introduction

Terminalia arjuna is the plant that belongs to the genus Terminalia, which belongs to the Combretaceae family and this plant is well distributed to the tropic and subtropics like warm temperature regions like Bangladesh, India, Srilanka etc. (Exell & Stace, 1966; Heywood et al. 2007). This plant is also known as koha or white marudah and it is used for many medicinal properties. One research work reported that the antioxidant action of aqueous extract of T. arjuna, which may play a role in the anti -carcinogenic activity by reducing the oxidative stress along with inhibition of anaerobic metabolism (Verma N., Vinayak M. 2009). There is no report of methanolic extract of this plant in respect of Polyphenolic compound like quercetin and anti-cancer drug like vincristine. However, phytochemical investigation revealed the anti-oxidant activity of the bark extract from Terminalia arjuna and its lethal bioassay test of two fraction (aqueous and ethyl acetate) for determining the cytotoxic activity of this plant.

Plant material

Plant sample of Terminali arjuna was collected from Dhaka in November 2017 from Dr. Md. Abdul Muhit, Assistant Professor, Department Of Clinical Pharmacy & Pharmacology, University Of Dhaka. Ref.No,CPP/DU/01-2016-TA.

Method

Bark of the plant was firstly separated from the plant. Then it was cut into small pieces and air dried for several days. The pieces were then oven dried for 24 hours at considerably low temperature (not more than 500C) to effect grinding. It was then ground into coarse powder. The coarse powder was then in air – tight container and kept in cool, dark and dry place for further use. The air dried and powdered plant material (500g) was submerged with methanol(2 litres) in an air tight, clean flat bottomed container for 15 days at room temperature with occasional stirring and shaking. It was then filtered through a fresh cotton plug and finally with a man N0.1filter paper. The volume of the filtrate was then reduced using a rotary evaporation at low temperature and pressure. The weight of the crude extract was 36.45 gm.

Solvent – solvent portioning

Solvent solvent partitioning was done using the protocol of the standard laboratory. The crude extract was dissolved in 600ml of
water. Then gently added 300 ml of Ethyl acetate for 4 times. The ethyl acetate layer was collected and the fraction of this was 10 g. Then the water layer fraction was collected about 19 g.

**Result**

Methanolic extracts were prepared to examine the total phenolic content, flavonoid concentration and antioxidant activity.

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**A.1 Total Phenolic count**

The total phenolic contents in the examined extracts was 449.8 mg GA/total phenolic contents in the plant extracts expressed in terms of gallic acid equivalent (mg of GA/g of extract) extract mg of GA/g of extract (X = 449.8 mg/ml).

**Total Flavonoid count**

The content of flavonoids was expressed in terms of quercetin equivalent (the standard curve equation: \( y = 0.0067x + 0.0132, r^2 = 0.999 \)), mg of Qu/g of extract. The concentration of flavonoids in plant extracts was 206.91 mg/g in methanolic extract. Concentrations of flavonoids in the plant extracts expressed in terms of quercetin equivalent (mg of Qu/g of extract) extract mg of Qu/g of extract (X = 206.91mg/ml).

**Anti-oxidant activity**

The capacity to neutralize DPPH radicals was found for methanolic extract which neutralized 50% (IC50) of free radicals at the concentration of 3.29 μg/ml, while the standard neutralizes 50% (IC50) of free radical at the concentration of 1.92 μg/ml. IC50 of Ascorbic acid is 1.92 μg/ml, where the IC50 of Terminalia Arjuna crude is 3.29 μg/ml.

**Table 1.1:** % of free radical scavenging capacity of Ascorbic acid and crude extract.

| No. | Ascorbic acid | Crude extract |
|-----|--------------|---------------|
|     | Conc (μg/ml) | Abs | % inhibit | Conc, (μg/ml) | Abs | % inhibit |
| 1   | 500          | 0.054 | 87 | 500 | 0.065 | 84 |
| 2   | 250          | 0.055 | 86 | 250 | 0.070 | 83 |
| 3   | 125          | 0.055 | 86 | 125 | 0.078 | 81 |
| 4   | 62.5         | 0.056 | 86 | 62.5 | 0.081 | 80 |
| 5   | 31.25        | 0.058 | 85 | 31.25 | 0.082 | 80 |
| 6   | 15.62        | 0.058 | 85 | 15.62 | 0.088 | 78 |
| 7   | 7.81         | 0.059 | 85 | 7.81 | 0.093 | 77 |
| 8   | 3.90         | 0.065 | 84 | 3.90 | 0.168 | 59 |
| 9   | 1.95         | 0.201 | 51 | 1.95 | 0.235 | 43 |
| 10  | 0.967        | 0.312 | 25 | 0.967 | 0.368 | 11 |
**Brine shrimp lethality bioassay**
This method is done to determine the cytotoxicity of the test samples from Terminalia Arjuna and their effectiveness on the cell to destroy them successfully.

**Table B.2: Results of the test samples of Terminalia Arjuna**

| Sample                | LC50(µg/ml) | Regression equation | R²  |
|-----------------------|-------------|---------------------|-----|
| Vincristine Sulphate (std) | 0.419       | Y = 30.404x + 61.071 | 0.9476 |
| MeOH crude            | 12.5        | Y = 0.2517x + 0.196  | 0.8951 |
| Aqueous fraction      | > 0.781     | Y = 0.143x + 0.6917  | 0.844  |
| Ethyl acetate fraction| > 0.781     | Y = 0.0685x + 0.8546 | 0.7961 |

**Table B.3: Effects of methanolic crude and it’s aqueous fraction on brine shrimp neupluii.**

| Conc. (µg/ml) | LogC | %Mortality | LC50 (µg/ml) | Vincristine sulphate |
|---------------|------|------------|--------------|----------------------|
|               |      |            |              | Conc.(µg/ml) | Log C | %Mortality | LC50 (µg/ml) |
| 400           | 2.602| 100        | 100          | 40                  | 1.602 | 100        | 0.419        |
| 200           | 2.301| 80         | 100          | 20                  | 1.301 | 100        |              |
| 100           | 2     | 60         | 100          | 10                  | 1.000 | 90         |              |
| 50            | 1.699| 50         | 100          | 5                   | 0.698 | 90         |              |
| 25            | 1.398| 50         | 100          | 2.5                 | 0.397 | 80         |              |
| 12.5          | 1.097| 50         | 80           | > 0.781             |       |            |              |
| 6.25          | 0.796| 40         | 80           | 1.25                | 0.096 | 70         |              |
| 3.125         | 0.495| 30         | 70           | 0.625               | -0.204| 60         |              |
| 1.563         | 0.194| 30         | 70           | 0.3125              | -0.505| 50         |              |
| 0.781         | 0.107| 20         | 70           | 0.15625             | -0.806| 30         |              |
| 0.107         | 0.011|            |              | 0.078125            | -1.107| 20         |              |
Figure B.1: Effect of Vincristine sulphate on brine shrimp neuplii.

Figure B.2: Effect of Crude sample on brine shrimp neuplii.

Figure B.3: Effect of Aqueous soluble partitionate of methanolic extract on brine shrimp neuplii.
Discussion

As we see, the amount of polyphenol is so high (449.8 mg/ml half of the total amount of 1 mg crude) and also the flavonoid is (206.91 mg/ml, we can say it is a very good antioxidant property containing plant part whose IC50 value (3.29 μg/ml) is also very good like standard (1.92 μg/ml).

The degree of lethality was directly proportional to the concentration of the extract ranging from significant with the lowest concentration (0.781 μg/ml) to highly significant with the highest concentration (400 μg/ml). Maximum mortalities took place at a concentration of 400 μg/ml, whereas least mortalities were at 0.781 μg/ml concentration. In other words, mortality increased gradually with the increase in concentration of the test samples. LC50 obtained from the best line slope were 0.419, 12.5, >0.781 μg/ml for vincristine, crude and other fractions respectively.

In comparison to positive control (vincristine) the cytotoxicity exhibiter by ethyl acetate and aqueous fraction of methanolic extract was highly significant. On the other hand, crude extract demonstrated promising activity.

Conclusion

The methanolic extract of Terminalia arjuna shows a great antioxidant activity almost like the standard ascorbic acid. It also has very good amount of phenol and flavonoid.

In the brine shrimp lethality bioassay, in comparison to positive control (vincristine sulphate) the cytotoxicity exhibited by the ethyl acetate fraction and aqueous fraction of the methanolic extract was highly significant.

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