Phytochemical screening, estimation of phenolic, flavonoid, ascorbic acid content and free radical scavenging assay of the aqueous bark extract of *Terminalia arjuna*

Yogyta Kumari[1] and Sadhna Jain[1*]

ABSTRACT

Since time immemorial the bark of Arjuna tree *(Terminalia arjuna)* has been used in the treatment of a variety of cardiovascular disorders. Many of these cardiovascular disorders stem from oxidative stress, therefore in this study we have tried to investigate the antioxidant properties of the aqueous bark extract of T. arjuna. The phytochemical screening of the bark extract was performed for identification of the essential phytochemicals present in it and there after the antioxidant strength was estimated by quantifying the amount of the major antioxidants present in plants, that is, phenols, flavonoids and ascorbic acid. Free radical scavenging assay was performed using DPPH free radical and the IC_{50} was estimated. On phytochemical screening the bark extract was found to contain alkaloids, flavonoids, saponins, phenols, sterols and tannins. The total phenol content was found to be 114.5 mg gallic acid equivalent per gram of bark. The total flavonoid content was found to be 8.9 mg of quercetin equivalent per gram of bark. The ascorbic acid content was found to be 190 mg per gram of bark. The free radical scavenging assay was performed using DPPH free radical and the IC_{50} was found to be 28.69μg/ml.

Keywords: *Antioxidants, Flavonoids, Phenolics, DPPH*

1. Introduction

Terminalia arjuna, commonly known as Arjuna is a tree belonging to the Combretaceae family. Different parts of the tree are used as ayurvedic remedies and it has been mentioned since the Vedic period in many ancient Indian medicinal texts (Dwivedi & Chopra, 2014) for its cardio protective properties. It is distributed throughout the greater part of India, Burma and Sri Lanka (Jain *et al.*, 2009). The plant is used traditionally for the treatment of a variety of ailments. *T. arjuna* has been found to be a very good hypo cholesterolaemic, hypo lipidemic, anti-coagulant, anti-hypertensive, anti-thrombotic, antiviral, antifungal and antibacterial agent (Jain *et al.*, 2009). Different parts of the tree have been investigated for the presence of phytoconstituents and pharmacological activities (Jain *et al.*, 2009). Particularly the bark of the Arjuna tree is known for its beneficial effects in cardiovascular disorders. Experimental studies have revealed that its bark possesses significant inotropic and hypotensive effect, increasing coronary artery flow and protecting myocardium against ischemic damage (Jain *et al.*, 2009).

Many of the cardiovascular disorders are thought to stem from oxidative stress. Oxidative stress is caused by an imbalance between the damage

1 *Shaheed Rajguru College of Applied Sciences for Women, University of Delhi*
* *Corresponding Author ✉ jainsadhna2015@gmail.com*

caused by reactive oxygen species (ROS) and the system's ability to repair that damage by readily detoxifying those reactive species. This results in base damage as well as strand breaks in DNA (Saxena P. Jain S., 2018). Oxidative stress in cardiac and vascular myocytes describes the injury caused to cells resulting from increased formation of ROS and/or decreased antioxidant reserve. The deleterious effects of ROS are mainly due to abilities of ROS to produce changes in sub-cellular organelles and induce intracellular calcium overload. Although the cause effect relationship of oxidative stress with any of the cardiovascular diseases remains to be elucidated, increased formation of ROS indicating the presence of oxidative stress has been observed in a number of cases. Furthermore, antioxidant therapy has been proven to be beneficial in hypertension, atherosclerosis, ischemic heart disease, cardiomyopathies and congestive heart failure. The existing evidence support the view that oxidative stress may play a crucial role in cardiac and vascular abnormalities in different types of cardiovascular diseases and that the antioxidant therapy may prove beneficial in combating these problems (Dhalla *et al.*, 2000).

The aim of this study was to get an estimate of the antioxidant strength of the aqueous bark extract of Arjuna tree, which is commonly used as a cardio-protective agent. The aqueous extract was taken to ensure its use as a nutraceutical.

2. Methodology

Arjuna bark was collected from arjuna trees. The bark was dried in shade and crushed using a mortar and pestle. It was then ground using a grinder. This powder was then used in preparing the bark extract as given in materials and methods. The bark was then screened for the presence of alkaloids, flavonoids, saponins, sterols, tannins and phenolics. After this quantitative test for phenols, flavonoids and ascorbic acid was performed. This was followed by a free radical scavenging assay using DPPH(1,1-diphenyl-2-picrylhydrazyl) free radical.

3. Materials and methods

Plant Material and sample preparation

Bark extract: 1 gram of bark was dissolved in 50 ml of distilled water and left overnight in an incubator shaker at 37°C, the extract was then filtered using filter paper and used. The concentration of extract prepared was calculated to be 0.02g/ml.

Chemicals used: Iodine, Potassium iodide, Sodium hydroxide, Hydrochloric acid, Ferric chloride, Chloroform, Sulphuric acid, Gallic acid, Folin-ciocalteu's reagent, Sodium carbonate, Sodium nitrite, Aluminium chloride, Sodium hydroxide, Trichloroacetic acid, 2,4-dinitrophenylhydrazine, Thiourea, Copper sulphate, Ascorbic acid.

Equipment: Incubator shaker, Spectro-photometer, Hot water bath, Ice bath.

Phytochemical screening

Test for alkaloids

Mayer's test (Santhi & Sengottuvel, 2016): To the aqueous extract added few drops of Mayer's reagent along the sides of the test tube. Presence of yellow creamy precipitate indicates presence of alkaloids.

Test for flavonoids

Alkaline reagent test: 2ml of 2% sodium hydroxide mixture was mixed with the extract, this turned the extract yellow in colour. Addition of 2 drops of dilute acid makes the solution colourless.

Test for saponins

Foam test (Santhi & Sengottuvel, 2016): 0.5 ml of the extract was vigorously shaken with 5 ml of water. Appearance of persistent foam indicates the presence of saponins.

Test for sterols

Salkowski test (Bargah, 2015): To 2 ml of extract, 2 ml of chloroform and 2ml of concentrated sulphuric acid was added leading to formation of a red ring at the interface of chloroform and acid layer.

Test for tannins

Ferric chloride test (Nandedkar& Mulani, 2016): To 2 ml of the extract few drops of 10% ferric chloride solution was added. Appearance of greenish-black colour indicates the presence of tannins.

Test for phenolics

Neutral ferric chloride test (Cs *et al.*, 2014): was performed. To 2 ml of bark extract few drops of neutral ferric chloride was added. The appearance of a dark violet colour complex indicates the presence of phenolics.

Estimation of total phenol content (TPC)

The total phenol content was estimated using folinciocalteu method (Saxena P. Jain S., 2018), using gallic acid as standard. 1ml aliquots of different concentrations of gallic acid (0, 20, 40, 60, 80, 100 µg/ml) were prepared and the volume was made 5ml using distilled water. 0.5 ml of FC reagent was added to this followed by 5 minutes incubation (Georgé *et al.*, 2005). After incubation 1.5ml of sodium carbonate and 3 ml of water was added. This solution was incubated for 2 hours at room temperature and the absorbance was recorded at 750nm. Standard curve was plotted using gallic acid as standard and the phenol content of the bark extract reported in milligrams of gallic acid equivalent per gram of bark.

Estimation of total flavonoid content (TFC)

The total flavonoid content was estimated (Saxena P. Jain S., 2018) using aluminium chloride colorimetric assay using quercetin as standard. 1 ml aliquots of different concentrations of quercetin (100, 200, 300, 400, 600, 800, 1000 µg/ml) were pipetted into test tubes. To this 300µl of 5% sodium nitrite was added, followed by addition of 300µl of 10% aluminium chloride and 2ml of 1 M sodium hydroxide. To this, 2.4ml of water was added to make the total volume 10ml. This solution was incubated for 1 hour and the absorbance of the sample was measured at 510 nm. Standard curve was plotted and the flavonoid content of the bark extract was reported in milligrams of quercetin equivalent per gram of bark.

Estimation of ascorbic acid content

Ascorbic acid quantity was estimated using 2,4-dinitrophenylhydrazine method. 20µg/ml ascorbic acid was prepared in 5% trichloroacetic acid. Pipetted 0.5ml,1ml,1.5ml, 2ml and 2.5ml of ascorbic acid into 5 test tubes and made the volume 3 ml using 5% trichloroacetic acid to make different concentrations of ascorbic acid. The Arjuna extract sample was also diluted in 5% trichloroacetic acid. To the standard ascorbic acid samples and Arjuna sample added DTC reagent (prepared using 2,4-dinitrophenyl hydrazine, thiourea and copper sulphate) incubated the test tubes for 1 hour at 60°C in a water bath and then immediately transferred to ice bath for 15 minutes. Then added 5 ml of 9 N sulphuric acid and allowed the solution to stand at room temperature for 20 minutes. Took the absorbance readings at 540 nm and plotted the standard curve.

Estimation of free radical scavenging activity using DPPH free radical radical (Musa et. al 2016)

Prepared different concentrations of the extract in water. To this added 1ml of DPPH and incubated in dark for 30 minutes. After 30 minutes recorded the absorbance at 517 nm and calculated the percentage inhibition of each sample using the formula $((A_C - A_T)*100) / A_C$ where A_C is the absorbance of control (only DPPH) and A_T is the absorbance of test (varying concentrations of extract sample with DPPH) at 517 nm. Plotted a curve between concentration and percentage inhibition and calculated the inhibitory concentration 50 (IC_{50}). The IC50/half maximal inhibitory concentration is that concentration of the bark extract that reduces fifty percent of the DPPH free radicals present in the sample.

4. Result

Phytochemical screening

According to the results obtained in phytochemical screening the aqueous bark extract of *T. arjuna* contains alkaloids, flavonoids, saponins,

Table-1: Result of Phytochemical Screening

Serial No.	Compound	Present/Absent
1	Alkaloid	Present
2	Flavonoid	Present
3	Saponins	Present
4	Sterols	Present
5	Tannins	Present
6	Phenolics	Present

sterols, tannins and phenolics. All of these results except for sterols have also been shown by Akhter *et al*. (Akhter *et al*., 2012). Presence of sterols has been shown in the aqueous extract by Tiwari *et al.* (Tiwari *et al., 2017).*

Estimation of total phenol content

The total phenol content was estimated using folin-ciocalteu method with gallic acid as standard. The equation of the standard curve was found to be (y = 0.010x + 0.094) and coefficient of determination (R^2) = 0.999. The total phenolic content of the Arjuna bark extract was found to be 114.5mg of gallic acid equivalent per gm of bark.

Estimation of total flavonoid content

The total flavonoid content was estimated using aluminium chloride colorimetric assay using quercetin as standard. The equation of the curve was found to be (y = 0.001x – 0.005) and coefficient of determination (R^2) = 0.983. The total flavonoid content of the Arjuna bark extract was found to be 8.9mg of quercetin equivalent per gram of bark.

Estimation of ascorbic acid content

Ascorbic acid was estimated using dinitrophenyl hydrazine method using ascorbic acid as standard. The equation of the curve was found to be (y = 0.005x + 0.016) and the coefficient of determination (R^2) = 0.956. The ascorbic acid content was found to be 190mg per gram of Arjuna bark.

Table 2: Result of quantitative analysis of the aqueous extract of the bark of T. arjuna.

Serial No.	Phytochemical	Amount present
1	Phenol	114.5 mg of gallic acid equivalent per gram of bark
2	Flavonoid	8.9 mg of quercetin equivalent per gram of bark
3	Ascorbic acid	190 mg of ascorbic acid per gm of arjuna bark

The phenol concentration was found to be 114.5 mg of gallic acid equivalents per gram of bark, the flavonoid concentration was found to be 8.9 mg of quercetin equivalent per gram of bark and 190 mg of ascorbic acid per mg of bark.

Estimation of free radical scavenging activity using DPPH free radical

The free radical scavenging activity of the extract was studied by its ability to reduce DPPH (1,1-diphenyl-2-picrylhydrazyl), a stable free radical and any molecule that can donate an electron or hydrogen to DPPH, can react with it and thereby bleach the DPPH absorption (Akhter *et al.* 2012). DPPH is a purple colour dye having absorption maxima at 517 nm and upon reaction with a hydrogen donor its purple colour fades or disappears due to conversion to 2,2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance (Akhter *et al.* 2012).

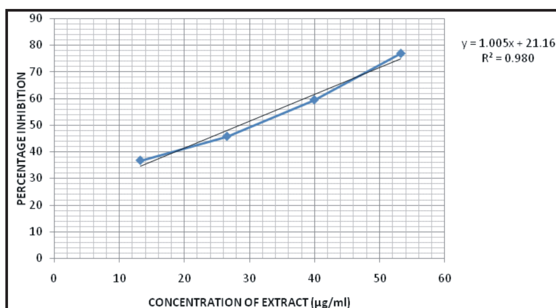

Figure 1: Graph between percentage inhibition and concentration of extract.

The graph has been plotted between the percentage inhibition of free radicals by bark extract and the corresponding concentration of bark extract used. We can observe from the graph that as the concentration of extract used increases, the percentage inhibition also increases. The concentration of the extract that inhibits 50% of free radicals in the sample is found out from the graph to be 28.69 μg/ml.

The bark extract showed strong free radical scavenging activity in a concentration dependent manner. The equation of the inhibition curve obtained (Figure 1) was found to be

$y = 1.005x + 21.16$ and $R^2 = 0.98$, the IC_{50} was found to be 28.69μg/ml of the aqueous extract.

From the above results we can conclude that the Arjuna bark extract is quiet rich in antioxidants such as phenols and ascorbic acid and has a very strong free radical scavenging activity. This strong antioxidant property is an important determinant of the various health benefits of *Terminalia arjuna* including its cardio-protective activity.

5. Discussion

Arjuna is a commonly used cardioprotective agent. Several theories have been proposed for its mechanism of action; however, none till date have been supported by firm proof. According to a study by Kapoor *et al*. (Kapoor *et al*., 2014) T. arjuna exerts a number of pleiotropic effects which contribute to its overall effectiveness, one of them being its antioxidant property. In this study we try to explore the antioxidant property of the aqueous bark extract of Arjuna, the form in which it is commonly used for as a medicine (Maulik *et al*., 2016). Previously similar studies have been done but using solvents other than water (Chatha, 2014; Shahriar *et al*., 2012; Sultana *et al*., 2007).

Besides the commonly known antioxidants we have also estimated the levels of ascorbic acid present in the bark extract which was not quantitated previously. On study of the bark extract it is found that ascorbic acid is the most abundant antioxidant found in the plant, followed by phenols and flavonoids. The abundance of antioxidants is reflected in the strong free radical scavenging activity of the bark extract.

6. Acknowledgement

The authors thank the Department of Biochemistry, Shaheed Rajguru College of Applied Sciences for Women for providing all the necessary material and intellectual support for completing this study.

7. References

1. Akhter, S., Haque, A., Shahriar, M., & Bhuiyan, M. (2012). Phytochemical Screening, Antibacterial, Antioxidant and Cytotoxic Activity of the Bark Extract of Terminalia Arjuna. *European Journal of Scientific Research*, *86*, 543–552.
2. Bargah, R. kumar. (2015). PreliminWary test of phytochemical screening of crude ethanolic and aqueous extract of Moringa pterygosperma Gaertn. *Journal of Pharmacognosy and Phytochemistry*, *4*(1), 07–09.
3. Chatha, S. A. S. (2014). Bioactive Components and Antioxidant Properties of Terminalia arjuna L.Extracts. *Journal of Food Processing & Technology*, *05*.
4. Cs, V., Vb, H., Sr, S., V, V., Nm, K., & Pg, L. (2014). Comparative preliminary phytochemical analysis of ethanolic extracts of leaves of Olea dioica Roxb., infected with the rust fungus Zaghouania oleae (E.J. Butler) Cummins and non-infected plants. *Journal of Pharmacognosy and Phytochemistry*, *3*(4), 69–72.
5. Dhalla, N. S., Temsah, R. M., & Netticadan, T. (2000). Role of oxidative stress in cardiovascular diseases. *Journal of Hypertension*, *18*(6), 655–673.
6. Dwivedi, S., & Chopra, D. (2014). Revisiting Terminalia arjuna—An Ancient Cardiovascular Drug. *Journal of Traditional and Complementary Medicine*, *4*(4), 224–231.
7. Georgé, S., Brat, P., Alter, P., & Amiot, M. J. (2005). Rapid determination of polyphenols and vitamin C in plant-derived products.

Journal of Agricultural and Food Chemistry, *53*(5), 1370–1373.

8. Jain, S., Yadav, P. P., Gill, V., Vasudeva, N., & Singla, N. (2009). Terminalia arjuna a sacred medicinal plant: Phytochemical and pharmacological profile. *Phytochemistry Reviews*, *8*(2), 491–502.

9. Kapoor, D., Vijayvergiya, R., & Dhawan, V. (2014). Terminalia arjuna in coronary artery disease: Ethnopharmacology, pre-clinical, clinical & safety evaluation. *Journal of Ethnopharmacology*, *155*(2), 1029–1045.

10. Maulik, S. K., Wilson, V., Seth, S., Bhargava, B., Dua, P., Ramakrishnan, S., & Katiyar, C. K. (2016). Clinical efficacy of water extract of stem bark of Terminalia arjuna (Roxb. ex DC.) Wight & Arn. in patients of chronic heart failure: A double-blind, randomized controlled trial. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, *23*(11), 1211–1219.

11. Musa, K. H., Abdullah, A., & Al-Haiqi, A. (2016). Determination of DPPH free radical scavenging activity: Application of artificial neural networks. *Food Chemistry*, *194*, 705–711.

12. Nandedkar, P. H., & Mulani, R. M. (2016). Phytochemicals and HPTLC studies of methanolic extract of different germplasms of Cordia dichotoma Frost f. *Journal of Pharmacognosy & Phytochemistry*, *5*(4), 06–12.

13. Santhi, K., & Sengottuvel, R. (2016). Qualitative and Quantitative Phytochemical analysis of Moringa concanensis Nimmo. *International Journal of Current Microbiology and Applied Sciences*, *5*(1), 633–640.

14. Saxena P. Jain S. (2018). Estimation of phenolic content, flavonoid content, antioxidant property and alpha amylase inhibition activity of pomegranate (PUNICA GRANATUM) and kinnow (hybrid of citrus nobilis and citrus deliciosa). *World Journal of Pharmaceutical Research*, *7*(2), 817–826.

15. Shahriar, M., Akhter, S., Hossain, M., Haque, A., & Bhuiyan, M. (2012). *Evaluation of in vitro antioxidant activity of bark extracts of Terminalia arjuna*.

16. Sultana, B., Anwar, F., & Przybylski, R. (2007). Antioxidant activity of phenolic components present in barks of Azadirachta indica, Terminalia arjuna, Acacia nilotica, and Eugenia jambolana Lam. Trees. *Food Chemistry*, *104*(3), 1106–1114.

17. Tiwari, A., Singh, R., & Tripathi, R. C. (2017). Phytochemical Screening and Antibacterial Activity of *Terminalia arjuna*. *Research Journal of Pharmacology and Pharmacodynamics*, *9*(3), 147.