

RESEARCH PAPER

Estimation of Total Phenolic Content and Antioxidant Activity of Methanolic Extracts of Leaf, Bark and Fruit of *Averrhoa bilimbi* Linn

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Abstract

Background: In folk medicine, *Averrhoa bilimbi* Linn. (Oxalidiaceae) is widely used and most parts of the plant are utilized for medicinal purposes. A review of the literature on this species turned up no information about the phenolic content and antioxidant activity of the bark of the bilimbi plant and comparative study of antioxidant activity among the leaf, bark and fruit of *A. bilimbi* linn.

Objective: This research aimed to estimate the phenolic content and anti-oxidant properties of leaf, bark, and fruit methanolic extracts of *A. bilimbi* Linn. and make a comparative study among them.

Methods: The total phenolics content was determined according to the Folin- Ciocalteu method and antioxidant activity was assessed by using DPPH radical scavenging assay.

Results: The methanolic extract of bark demonstrated the highest phenolic content (180.5 mg/gm GAE). The greatest DPPH radical scavenging was shown by bark methanolic extract with IC₅₀ value of 111.80 µg/ml.

Conclusion: The study indicates that methanolic extracts of *A. bilimbi* are perhaps a potent origin of natural antioxidant that may have large significance as a remedy in inhibiting or showing the advance of aging and relevant oxidative stress associated degenerative illness.

Keywords: Phenolic, Antioxidant, *Averrhoa bilimbi*; Gallic acid, DPPH.

Introduction

By scavenging free radicals, an antioxidant lowers the risk of degenerative illnesses by inhibiting or delaying the oxidative deterioration of an organism's cells. Atypical production of free radicals can lead to a number of illnesses such as cancer, cardiovascular problems, liver diseases, kidney diseases, autoimmune disorders, neurodegenerative diseases, aging etc. Various medicinal plants contain natural antioxidants, which are in charge of preventing oxidative stress's detrimental effects. Polyphenols and flavonoids containing plants serve as scavengers of free radicals, lessen oxidative stress, and may be a substitute remedy for various diseases. Various phytochemicals, including tannins, lignins, glycosides, alkaloids, phenols, and flavonoids are present in Plant and plant-based products. The most common plant

components that exhibit antioxidant properties are flavonoids and phenols.¹ Natural antioxidants such as phenols and flavonoids obtained from plant source are popular due to the side effects of synthetic antioxidants.² Numerous medicinal plants have been screened for their antioxidant and other pharmacological activities. Novel and potent antioxidants may come from the medicinal plants used in traditional medicine to treat oxidative stress and other associated conditions.

Averrhoa bilimbi Linn. is well known as medicinal plant of the Oxalidaceae family, it is frequently referred to as "Bilimbi". It is native to Malaysia and Indonesia and also found throughout the Bangladesh. The plant is used in folk medicine for its potential use in the treatment of inflammatory diseases, cough, cold, syphilis, hypertension, diabetes, piles and scurvy, whitlows, hepatitis, diarrhoea, pyrexia, beri-beri, biliousness, rectal bleeding, internal hemorrhoids, control obesity, itching, pimples, swellings of mumps, rheumatism, eruptions of skin, bites of toxic organism,

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rectal inflammations, cough and thrush.³⁻⁸ Various studies have indicated that the leaf and fruit of *A. bilimbi* have been reported to possess analgesic, cytotoxic, hepatoprotective, hypoglycaemic, hypolipidemic, hypotensive, nephrotoxic, pediculicidal, thrombolytic, antimicrobial, anticoagulant, anti-fertility, antimalarial, anthelmintics, and wound healing properties.⁹⁻¹¹ The antioxidant activity of leaf and fruit of *A. bilimbi* Linn. plant has been reported; however, antioxidant activity of bark and information on the relationship between antioxidant activity and phenolic content is not available. The objective of this study was to estimate the total phenolic contents and evaluate antioxidant activity in methanolic extracts of leaf, fruit and bark of *A. bilimbi* plant and explore relationship between phenolic content and antioxidant activity.

Materials and Methods

Collection and Extraction of Plant Materials: The plant samples were collected from Mohakhali and Tejgaon the district of Dhaka of Bangladesh in March 2017 and identified by Bangladesh National Herbarium taxonomists as parts of *Averrhoa bilimbi* Linn. Fruits, leaves, and stem bark that were collected were dried, pulverized and extracted with methanol (95%) by maceration, and then filtered and evaporated to concentrate the crude extract.

Estimation of Total Phenolics Content by Folin-Ciocalteu method: In Folin-Ciocalteu method, Folin-Ciocalteu Reagent (FCR) is oxidant and gallic acid is standard to make the calibration curve.¹²⁻¹³ Polyphenols containing specimens are reduced by the FCR with formation a blue color. Phosphomolybdate and phosphotungstate are the constituents of the reagent used to estimate antioxidant capacity of phenolic and polyphenolic compounds in colorimetric assay.¹⁴ It acts by estimating the quantity of the testing material required to resist the oxidation of the reagent.¹⁵

0.025 gm gallic acid or extracts were taken in methanol and adjusted the volume up to 5 ml with methanol to prepare 5µg/µl concentration of each solution. These solutions were thought as standard or extract stock solutions. The stock solution was used to make the experimental concentrations 6.25, 12.5, 25, 50, 100 and 200µg/µl.

One (1.0) ml extract (200 µg/ml) or standard of various concentration solution was taken in test tube. Then 5

ml Folin-Ciocalteu (Diluted 10 fold or diluted with water 1:10 v/v) reagent was mixed. 4 ml Na₂CO₃ solution (7.5%) was mixed to the same test tube. Incubated the standard solutions containing test tubes for half-hour at 20°C and incubated the extract solution containing test tubes for 1 hour at 20°C to finish the reaction. At 765 nm absorbance was measured against methanolic blank by a spectrophotometer. Total phenolic content in extracts was estimated as follows:

$$C = (c \times V)/m$$

Where C= total phenolic quantity, mg/gm plant extract, in GAE, c = gallic acid concentration established from the calibration curve (mg/ml), V = extract volume in ml, m = crude plant extract weight in gm.

Evaluation of Antioxidant Activity by DPPH Radical Scavenging Assay: The electron-transfer-based DPPH free radical technique is an antioxidant assay. DPPH is an oxidizing agent that accept electron and oxidize the other substances whereas antioxidant is a reducing agent that donate electron or hydrogen. DPPH is a stable (in pulvis form) free radical with red colour and forms violet colour in solution which turns pale yellow or colorless when scavenged. The free radical scavenging activity is shown by the DPPH assay using this character. Antioxidants reduce DPPH to DPPH-H by reacting with it and as a result the absorbance decreases. The amount of discoloring shows the neutralizing potential of the antioxidant substances or extracts with regard to hydrogen donate capability.¹⁶⁻¹⁷

0.025 gm ascorbic acids or extracts were taken in methanol and adjusted the volume up to 5 ml with methanol to prepare 5µg/µl concentration of each solution. These solutions were thought as standard or extract stock solutions.

Serially dilute the stock solution to obtain the concentration of 800µg/mL, 400 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL. Marked each test tube containing 1ml dilute the stock solution of each concentration. 2 mL 0.004% DPPH solution was mixed in each test tube and adjusted the final volume up to 3 ml. Incubated the solution for a period of 30 minutes at room temperature in a dry and dark space. At 517 nm absorbance was estimated against dilute extract solution in the solvent. The following formula was used to determine the % inhibition of the DPPH radical:

% inhibition = (1 - absorbance of sample/absorbance of control) × 100

Result

Total phenolic quantity of crude extracts of various parts of Bilimbi was measured with Folin's phenol reagent and was revealed as Gallic acid equivalents (GAE) per gram of plant extract. The Gallic acid ($y = 0.0106x + 0.050$; $R^2 = 0.999$) standard curve (Figure 1)

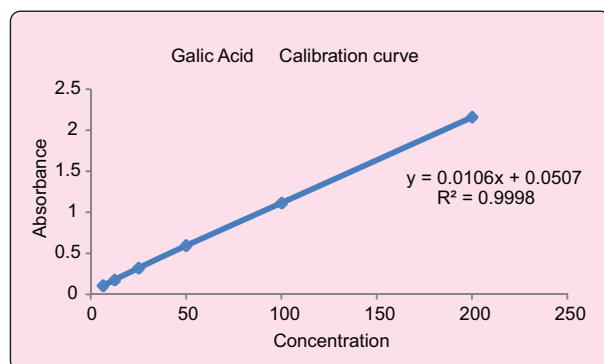


Figure 1: Gallic Acid Calibration Curve

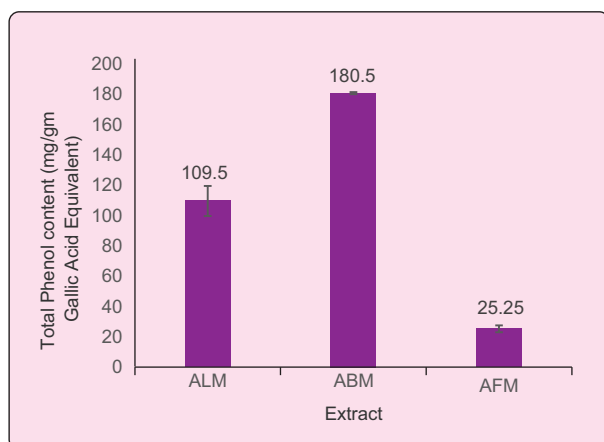


Figure 2: Total phenol contents of the various extracts of *A. bilimbi* Linn.

was used to measure the total phenolic amounts of the test samples. The phenolic content in methanolic (95%) extract of stem bark of *A. bilimbi* was higher (Table I).

Table I: Total phenol contents of the various extracts of *A. bilimbi* Linn.

Extract	Total phenol quantity (mg/gm, GA equivalents)
ALM	109.5±9.89
ABM	180.5±0.71
AFM	25.25±2.25

Values are the mean of duplicate experiments and represented as mean ± SD.

The result of phenolic contents can be shown in the following order: ABM>ALM>AFM (Table I). The abbreviations ALM, ABM, and AFM stand for methanolic extracts of *Averrhoa bilimbi* leaf, bark, and fruit respectively.

The potency of the drug is correlated with the IC_{50} value. A higher activity of the plant extract is indicated by a lower IC_{50} value.¹⁸ In this research, the free radical neutralizing action of the methanolic extract of the bark of *A. bilimbi* was found to be highest having IC_{50} value of 111.80 $\mu\text{g/ml}$ and lowest action was demonstrated by the methanolic extract of the fruits of *A. bilimbi* with IC_{50} being 391.39 $\mu\text{g/ml}$. The *A. bilimbi* leaves methanolic extract showed moderate free radical neutralizing action with the IC_{50} value 223.57 $\mu\text{g/ml}$, as compared to the standard, i.e., ascorbic acid ($IC_{50} = 11.86 \mu\text{g/ml}$). IC_{50} values of the various extracts of *A. bilimbi* are shown in the Table II.

Table II: IC_{50} values of the various extracts in DPPH scavenging assay

Sample/Standard	IC_{50} ($\mu\text{g/ml}$)
ALM	223.57
ABM	111.80
AFM	391.39
Ascorbic Acid	11.86

DPPH radical neutralizing capability of the extracts was shown to reduce as follows: Ascorbic Acid> ABM> ALM>AFM.

Discussion

Phenolics compounds are commonly present in the plant. They include flavonoids (flavones, isoflavones, and flavonones), anthocyanins and catechins, and have free radical neutralizing action. The antioxidant characteristics of polyphenols come from their strong responsiveness as electrons or hydrogen atoms donors.¹⁹ The oxidation reactions play an important role in atherogenesis.²⁰ Moreover, plasma LDL cholesterol concentration is reversely proportional to quercetin intake.²¹ Polyphenols containing compounds reduce the production of atherosclerotic plaques and decrease arterial stiffness by suppressing oxidation of LDL.²¹⁻²³ The quantity of total phenolics

was found vary in various extracts compared with gallic acid equivalent. The highest total phenolic content was found from methanolic extract of the bark of *A. bilimbi* and lowest from fruit methanolic extract of *A. bilimbi*. The methanolic extract of the leaf of *A. bilimbi* demonstrated moderate total phenolic content (Table I).

DPPH anti-oxidant test is founded on the capability of DPPH to decolorize in presence of anti-oxidants.²⁴ As this assay depends on electron-transfer, so the antioxidant substances in the extracts of plant and the reference drug neutralized DPPH free radical by shifting either electrons or hydrogen atoms to DPPH, as a result altering from violet to pale yellow coloured diphenylpicrylhydrazine.²⁵ The rate of decolorization showed the neutralizing capacity of the plant extracts with regard to donating capability of hydrogen which is quantitatively assessed from the changing in absorbance.²⁶ The mixing of plant extract with the solution of DPPH made a quick reduction in absorbance at 517 nm showing the excellent neutralizing capacity of plant extract.²⁵ In DPPH method, the crude extracts of *A. bilimbi* showed neutralizing of DPPH radicals in a process same to the reference standard (Figure 3).

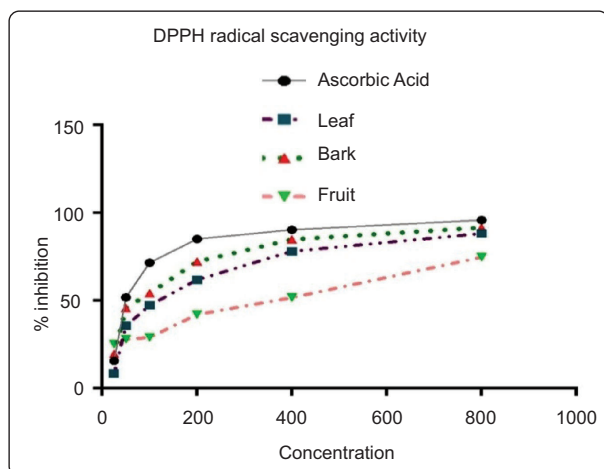


Figure 3: DPPH radical scavenging activity of the various extracts of *A. bilimbi* Linn.

The various extracts of *A. bilimbi* possess the capacity to donate electron among which methanolic extract of stem bark showed maximum potency (Table II). The Scavenging activity of DPPH radical is related to the quantity of phenolic compound of the plant extract and there exist a positive relationship between the phenolic amounts and DPPH neutralizing capability of extracts of the plant. The *A. bilimbi* bark methanolic

extract exhibiting maximum DPPH neutralizing capacity (Table II) also possess highest phenolic quantity (Table I). From this study it is evident that higher the phenolic amounts lower the IC_{50} value, i.e., maximum the DPPH neutralizing ability of the extract of plant. The DPPH assay estimates the electrons or hydrogen atoms giving power of the extracts of plant to DPPH radical produced in solution.²⁷

The aqueous methanolic extracts of *A. bilimbi* Linn leaf, bark and fruit exhibited excellent antioxidant activity. The leaves and stem bark methanolic extracts showed higher action than the fruit extract. Phytochemical constituents chiefly phenolic compounds (Table I) of the methanolic extract of *A. bilimbi* may be contribute the total antioxidant activity.

Conclusion

Methanolic extracts in this investigation demonstrated strong antioxidant capability, indicating the biological activity of the samples. The antioxidant activity was evaluated using established *in vitro* models such as DPPH free radical scavenging assay. The methanolic extract of bark demonstrated highest phenolic content (180.5 mg/gm GAE). Greatest DPPH radical scavenging was shown by bark methanolic extract with IC_{50} value of 111.80 μ g/ml. The current study's findings indicate that the methanolic extracts of *A. bilimbi* leaf, bark and fruits may be source for antioxidant agent. The precise mechanism(s) and/or active principle(s) of antioxidant action require further investigation.

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References

- Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Dietary polyphenols and the prevention of diseases. *Crit Rev Food Sci Nutr.* 2005; 45: 287–306. DOI: 10.1080/1040869059096
- Zhong R-z, Zhou D-w. Oxidative stress and role of natural plant derived antioxidants in animal reproduction. *J Integr Agric.* 2013; 12:1826-38. DOI: 10.1016/S2095-3119(13)60412-8
- Jayaweera DMA. Medicinal plants (indigenous and exotic) used in Ceylon. Part IV. The National Science Council of Sri Lanka, Colombo, Sri Lanka; 1982. p. 166-67. Available from: catalogue.nla.gov.au/Record/826713
- Kirtikar KR, Basu BD. Indian medicinal plants. Volume 1. 2nd ed. 44, International Book Distributors, Dehradun, India; 1935. p. 443. ISBN: 10: 812128774X
- Ghani A. Medicinal Plants of Bangladesh. 2nd ed. The Asiatic Society of Bangladesh. Dhaka, Bangladesh; 2003. p.115-16. ISBN: 984-512-348-1
- Yusuf M, Chowdhury JU, Wahab MA, Bejom J. Medicinal Plants of Bangladesh, 1st ed. BCSIR Laboratories, Chittagong, Bangladesh;1994. p. 31. Available from: lib.icimod.org/record/4803
- Morton J. Fruits of warm climates. Miami, Florida, USA. 1987. p. 128-29. ISBN: 0-9610184-1-0
- Ali MR, Hossain M, Runa JF, Hasanuzzaman M. Preliminary cytotoxic activity of different extracts of *Averrhoa bilimbi* (fruits). *Int Curr Pharm J.* 2013; 2: 83-84. DOI: 10.3329/icpj.v2i3.13634
- Anitha Roy, Geetha RV, Lakshmi T. *Averrhoa bilimbi* Linn.- Nature's Drug Store-A Pharmacological Review. *Int J Drug Dev & Res.* 2011; 3: 101-06. Available from: www.researchgate.net/publication/282735763
- Alhassan AM, Ahmed QU. *Averrhoa bilimbi* Linn.: A review of its ethnomedicinal uses, phytochemistry, and pharmacology. *J Pharm Bioall Sci.* 2016; 8:265-71. DOI: 10.4103/0975-7406.199342
- Sarker MAM, Chowdhury AYSFUA. Analgesic effect of methanolic extracts of leaf, bark and fruit of *Averrhoa bilimbi* Linn. *Bangladesh Medical Res Counc Bull.* 2022; 48: 120-26. DOI: 10.3329/bmrcb.v48i2.62298
- Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J Agric and Food Chem.* 1998; 46: 4113-17. DOI: 10.1021/jf9801973
- Majhenic L, Skerget M, Knez Z. Antioxidant and antimicrobial activity of guarana seed extracts. *Food Chem.* 2007; 104: 1258-68. DOI:10.1016/j.foodchem.2007.01.074
- Singleton VL, Orthofer R, Lamuela-Raventos R M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* 1999; 299: 152-78. DOI: 10.1016/S0076-6879(99)99017-1
- Vinson J, Zubik L, Bose P, Samman N, Proch J. Dried fruits: excellent in vitro and in vivo antioxidants. *J Am Coll Nutr.* 2005; 24: 44-50. DOI: 10.1080/07315724.2005.10719442
- Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. u.-Technol.* 1995; 28: 25-30. Available from: dx.doi.org/10.1016/S0023-6438(95)80008-5
- Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from *Bauhinia terapotensis*. *J Nat Prod.* 2001; 64: 892-95. DOI: 10.1021/hp0100845
- Maisuthisakul P, Suttajit M, Pongsawatmanit R. Assessment of phenolic content and free radical scavenging capacity of some Thai indigenous plants. *Food Chem.* 2007; 100: 1409-18. DOI: 10.1016/j.foodchem.2005.11.032
- Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* 1997; 2: 152-59. DOI: 10.1016/S1360-1385(97)01018-2
- Ame SN, Shigrenaga MK, Hagen TM. Oxidants, antioxidants and degenerative diseases of ageing. *Proc Natl Acad of Sci USA.* 1993; 90: 7915-22. DOI: 10.1073/pnas.90.17.7915
- Arai Y, Wantanabe S, Kimira M, Shimoi K. Dietary intakes of flavonols, flavones and isoflavones by Japanese Women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. *J Nutr.* 2000; 130: 2243-50. DOI: 10.1093/jn/130.9.2243
- Moline J, Bukharovich IF, Wolff MS. Dietary flavonoids ad hypertension: is there a link? *Med Hypotheses.* 2000; 55: 306-09. DOI: 10.1054/mehy.2000.1057
- Duthie GG, Duthie SJ, Kyle JAM. Plant polyphenols in cancer and heart disease: implication as nutritional antioxidants. *Nutr Res Rev.* 2000; 13: 79-106. DOI: 10.1079/095442200108729016
- Kumarasamy Y, Byres M, Cox PJ, Jaspars M, Nahar L, Sarker SD. Screening seeds of some Scottish plants for free-radical scavenging activity. *Phytother Res.* 2007; 21: 615-21. DOI: 10.1002/ptr.2129
- Awah FM, Verla AW. Antioxidant activity, nitric oxide scavenging activity and phenolic contents of *Ocimum gratissimum* leaf extract. *J Med Plant Res.* 2010; 4: 2479-87. DOI:10.5897/JMPR10.262
- Mosquera OM, Correa YM, Buitrago DC, Nio J. Antioxidant activity of twenty five plants from Colombian biodiversity. *Mem Inst Oswaldo Cruz.* 2007; 102: 631-34. DOI: 10.1590/S0074-02762007005000066
- Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tementosa* Miller (Lamiaceae). *Food Chem.* 2005; 90: 333-40. DOI:10.1016/j.foodchem.2003.09.013