ejpmr, 2019,6(1), 102-106

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF BARK & LEAF EXTRACTS OF *BAUHINIA ACUMINATA*

Md. Raza Ul Karim[1]*, Tufikul Islam[1], Samiran Kumar Sadhu[2], Md. Manjur Rahman[1], Bhuiyan Md. Ashiqur Rahman[1] and Md. Nasir Uddin[1]

[1]Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.
[2]Department of Pharmacy, University of Khulna, Sher-E-Bangla Road, Khulna 9208, Bangladesh.

***Corresponding Author: Md. Raza Ul Karim**

Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

Article Received on 07/11/2018 **Article Revised on 28/11/2018** **Article Accepted on 19/12/2018**

ABSTRACT

The prime interest of the current study was to investigate the antioxidant & antimicrobial activity from the ethanolic extracts ofbark and leaf of the herb Bauhinia acuminata.Three antioxidant methods were followed to investigate antioxidant property. In total phenol content determination,both leaf and bark extracts showed the value of 29.00 mg/g and 32.00 mg/g as Gallic Acid Equivalent (GAE) respectively. In total Tannin content determination both leaf and bark extracts showed the value of 42.00 mg/g and 46.00 mg/g as Tannic Acid Equivalent (TAE) respectively.TheDPPH free radical scavenging activity ofboth leaf and bark extracts with IC_{50}is30.00µg/mL and 35.00µg/mL respectively. During antimicrobial activity both the bark and leaf extracts showed antimicrobial activities comparing with standard antibiotic Kanamycin. Further studies are needed to isolate active compounds.

KEYWORDS: Bauhinia acuminata, DPPH, Kanamycin.

INTRODUCTION

Healing with medicinal plants is parallel with the human being.[1] For thousands of years natural products have been used in traditional medicine.[2] According to World Health Organization, the global market of plants & its derivative products are estimated $83 billion and growing continuously. But the fact is disappointing that out of 3 lakhs plant species that exist in world, mere only 15% have been evaluated as medicine.[3] With the light of pharmacological activity and economic viability a recent scientific development of natural products are going on.[4] Chemical investigations of such natural products have lead to the discovery of important drugs such as aspirin, digitoxin, morphine, quinine and pilocarpine.[4] A great number of plants which are aromatic, spicy and medicinal in nature maintain antioxidant properties.[5] An important role of antioxidant is to increase the shelf life of food and reduces nutritional losses and formation of harmful substances.[6] Due to gradual increase of microbial resistant, cost and adverse effects of present antimicrobial agent development of new antimicrobial drug is prime need to treat infectious disease. Bioactive plant extracts serve for anti-infective drug discovery which are highly effective against microbes. Moreover, antibiotics obtained in this way have biological friendliness nature.[7]

Bauhinia acuminata belongs to the family of Fabaceae (Leguminosae), which possesses antioxidant[8] and antimicrobial[9] properties. Both leaf and barks of *Bauhinia acuminata* has been used traditionally various skin diseases, worms, tumors, diabetes, diuretic and demulcent.[10]

MATERIALS AND METHODS

Chemicals: Ethanol and Folin-Ciocalteu reagent were purchased from Merck, Germany. Sodium carbonate (Na_2CO_3), Potassium Acetate and H_2SO_4 (98%) were purchased from Merck (India) Limited. Gallic acid and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemicals, USA. Ascorbic acid was purchased from SD Fine Chem. Ltd., Biosar, India. All chemicals and reagents used were of analytical grade.

Collection of Plant Material

The bark and leaf of the plant was collected from Khan BahadurAshsanullah Hall, Khulna University, Bangladesh.

Preparation of Plant material & Extraction procedure: Bark and leaf of the plant were first washed with water to remove adhering dirt and then cut into small pieces and sun-dried for few days and then dried in a hot air oven (Size 1, Gallenkamp) at reduced temperature (not more than 50°C). Dried bark and leaf were grinded into coarse powder using high capacity grinding mill separately. Two clean flat-bottomed glass containers with 800 mLof 95% ethanol in each were taken and placed about 200gm ofpowered material of bark and leaf.Each container is then separately soaked

and kept for 15 days. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material.The filtrates were taken into beaker and the opening of beaker were wrapped by a sheet of aluminum foil to which perforation was done for evaporation of the ethanol &was kept in dry & cool place for several days. Then table fan was used until dried. It rendered concentrate of greenish black color. The concentrate was designated as crudeethanol extract of the bark extract and leaf extract of *Bauhinia acuminate.*[11]

Phytochemical Screening: The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents i.e. carbohydrates through molisch's test and fehling's test, flavonoids,glycoside, steroids, saponins through frothing test, tannins through Ferric chloride and Potassium dichromate test, alkaloids through mayer's test, hager's test, wagner test and dragendorff's test. These phytochemicals were identified from their respective characteristic color changes as stated in the standard procedures.[12]

Antioxidant Activity Evaluation

Total phenol content determination: Total phenolic content of the prepared ethanol extracts was determined by using modified Folin–Ciocalteu Reagent (FCR).[13] Half (0.5) mL of extract (1000 µg/mL) and the standard (gallic acid) of different concentrations (0.00, 100, 200, 300, 400 and 500 µg/mL) were taken into marked six test tubes. All test tubes were marked accordingly. Five (5) mL of Folin–Ciocalteu reagent solution (diluted to 10 fold) were taken in the test tubes followed by the addition of 4 mL of 7.5% sodium carbonate solution in each. The test tubes were incubated at 20°C (30 minutes for standard solutions, and 1 hour for extract solution). Absorbance at 765 nm was measured using a UV-Vis spectrophotometer (Shimadzu UV PC-1600) against a blank. Total phenol content of the fractions was expressed as Gallic acid equivalents (GAE).[14,15]

Determination of Total Tannin Content

The tannins were determined by slightly modified Folin and Ciocalteu method.[13] The standard (Tannic Acid) solution of six different concentrations (0.00, 100, 200, 300, 400 and 500 µg/mL) andthe extract (1000µg/mL) of 0.1 mL were taken in different marked test tubes. Then 7.5mL of distilled water, 0.5 mL of Folin Phenol reagent, 1 mL of 35% sodium carbonate solution were added and the volume was finally adjusted up to 10 mL with distilled water. The mixture was shaken well, kept at room temperature for 30 minutes and absorbance was measured using UV-Vis spectrophotometer (Shimadzu UV PC-1600) at 725 nm against a blank. Total Tannin content of the extracts was expressed as Tannic Acid Equivalent (TAE).

DPPH Free Radical Scavenging Assay

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of the plant extract was determined following the method described by Braca*et al.*[17] Two (2.0) mL extract of different concentrations (512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/mL) and standard (ascorbic acid) of different concentrations (512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/mL) were taken in different pre-marked test tubes. Then, 6 mL of 0.004% methanolic DPPH solution was added to each test tube. All the prepared test tubes with their contents were then incubated for 30 minutes at room temperature. Absorbance of each of the incubated solutions was determined at 517 nm using UV-Vis spectrophotometer (Shimadzu UV PC-1600) against a blank and IC_{50} value was calculated from the curve. (Fig.: 5).

Antibacterial Activity by Disc Diffusion Method

The antimicrobial activity of different extracts were determined by the disc diffusion method.[17] The bacterial strains used for the experiment were collected as pure cultures from the Microbiology Lab. of Pharmacy Discipline, Jahangirnagar University. Both Gram positive and gram negative organisms were taken for the test and they are listed in the following Table 1. Solutions of known concentration (50µg/mL) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs were then impregnated with 10µL of the test samples (500µg/ Disc) using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs (Kanamycin 30 µg/disc) and blank discs (impregnated with respective solvents 10µL) were used as a positive and negative control. These plates were then incubated at 37ºC for 24 h allowing maximum growth of the organisms. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in millimeter.

RESULTS

Table. 1: Microorganisms used in antimicrobial assay.

Gram Positive Bacteria	Gram Negative Bacteria
Staphylococcusaureus	Escherichiacoli
Staphylococcussaprophyticus	Shigelladysenteriae
Streptococcusagalactiae	Pseudomonassyringae
Staphylococcusepidermidis	Shigallaboydii
	Shigellasonnei
	Shigellaflexneri

Table. 2: Results of Phytochemical Screening of the extracts.

Phytochemical Group of Compounds	Result (Bark)	Result (Leaf)
Alkaloid	+	+
Carbohydrate	+	+
Flavonoid	+	+
Glycoside	-	+
Saponin	+	+
Steroid	-	-
Tannin	+	+

['+' sign indicates presence of phytochemical group of compounds while the '-' sign indicates absence of phytochemical group of compounds tested for]

Table. 3: Total phenolic, flavonoid contents, Total Tannin Contents&IC_{50}(mean ± SD) of extracts from bark and leaf of *B. acuminate.*

Plant Extracts	Total Phenolic Content (mg/gm GAE)	Total Tannin Content (mg/gm TAE)	DPPH Scavenging Assay IC_{50} µg/mL
leaf	29.00±0.184	42.00±0.00	30.00
bark	32.00±0.065	46.00±0.00	35.00

Table. 4: Anti-microbial screening of ethanol extracts of barkand Leafof *B. acuminate.*

Name of bacterial strains	Type of bacterial strain	Zone of inhibition (mm)			
		Standard Kanamyci n (30µg/disc)	*B. acuminata*(bark) 500µg/ disc	*B. acuminata*(Leaf) 500µg/ disc	Control
Pseudomonassps.	Gram (-ve)	30.18	11.66	8.36	0
Shigellaboydigius		18.71	14.15	10.32	0
Shigellasonnei		20.93	8.08	5.47	0
Shigellaflexineri		24.24	10.26	9.25	0
Escherichia coli		21.08	15.05	14.85	0
Staphylococcus epidermidis	Gram (+ve)	21.94	13.80	3.97	0
Staphylococcus pyogens		32.44	15.64	13.64	0
Staphylococcus aureus		28.36	8.99	7.54	0
Streptococcus agulectiae		30.84	12.60	12.06	0
Enterococcus faecalis		30.74	12.45	11.6	0

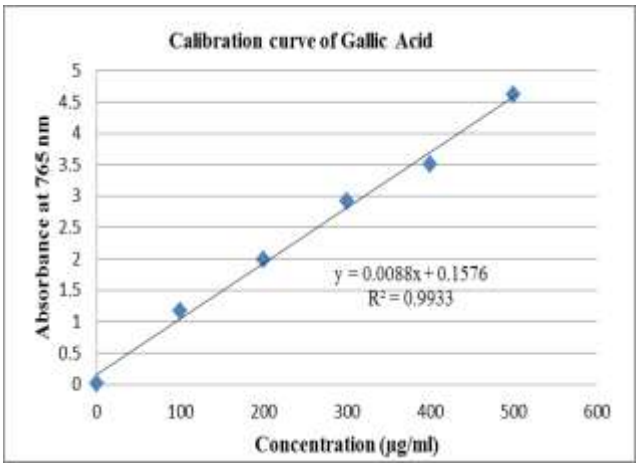


Fig. 1: The calibration curve of gallic acid to determine total Phenol content

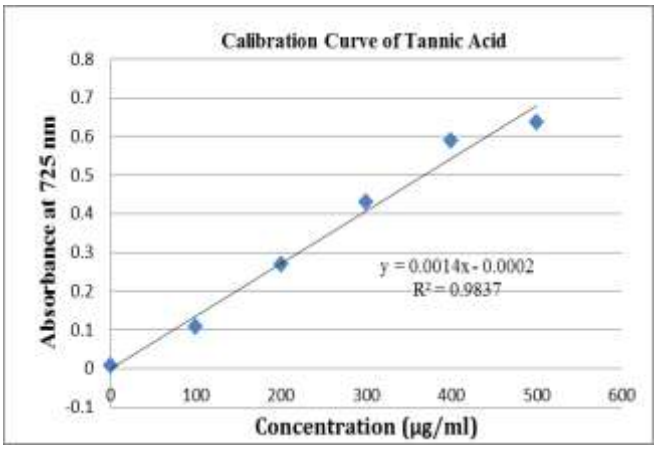


Fig. 2: The calibration curve of Tannic acid to determine total tannin content.

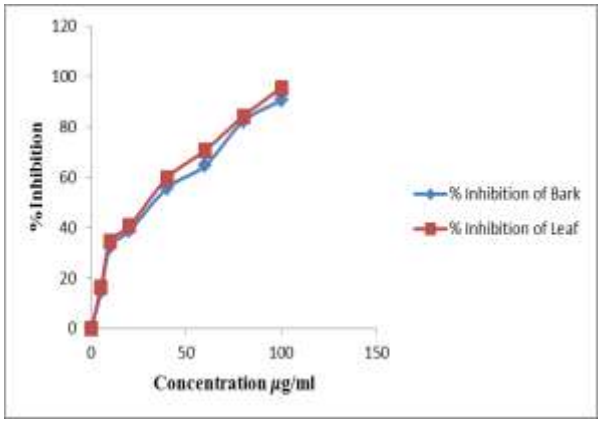


Fig. 3: DPPH radical scavenging activity of the different extracts of bark and leave.

Phytochemical screening*:* In the present study, various qualitative tests were done to detect the presence of different phytochemical compounds in ethanol extracts of the bark and leaf of *Bauhinia acuminata*. Phytochemical constituents in the plants are known to be biologically active compounds and they are responsible for different activities against diseases.[18] The results of the phytochemical testing are given in Table 2.

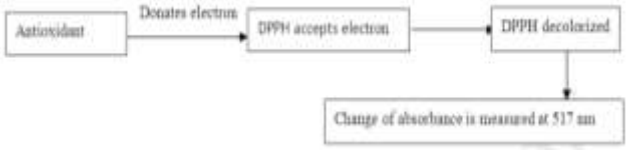
Antioxidant activity evaluation

Total phenol content determination: Phenolic compounds of plants have been said to account for most of the antioxidant activities of plant extracts.[19] They show antioxidant activities by preventing decomposition of hydroperoxides into free radicals or by inactivating the lipid free radicals.[20] The total phenolic compounds content of the test solutions were calculated using the calibration curve of the standard (Fig. 1)of Gallic acid ($y = 0.0106x + 0.0507$, $R^2 = 0.9998$)**.** The results were expressed as gallic acid equivalents (GAE) per gram of the extract. Ethanolic extract of bark and leafof *Bauhinia acuminate* were found to contain phenolsrespectively 32.00±0.065 and 29.00±0.184 mg/gm GAE (Table 3). (Table 3).

Determination of Total Tannin Content: Total tannin content of the different extracts bark and leaf of *Bauhinia acuminata*L were evaluated by the modifiedFolin method and was expressed as tannic acid equivalents (TAE) per gram of plant extract. Total tannin capacity of the test samples was calculated using the standard curve of tannic acid ($y = 1.3587x + 0.0001$; $R^2 = 0.9838$) (Fig. 3). Ethanolic extract of bark and leaf were found to possess the Total tannin content 46.00±0.00 mg/gm and 42.00±0.00 mg/gm TAE respectively (table 3).

DPPH Free Radical Scavenging Assay

To evaluate the radical scavenging activity of antioxidants, the DPPH free radical scavenging assay is justified as it is accurate & economical. Moreover its stable & non generating character makes as choice for assay.[21]



The IC_{50} values of the different extracts of *B. acuminata* are presented in the Table 3. The IC_{50} value of bark and leaf of extracts were found 35.00 μg/mL and 30.00 μg/mL respectively. The value is 7.50 μg/mL for the standard ascorbic acid. (Fig. 3).

The IC_{50} is inversely related to the antioxidant property of plant.[22] In the present study, extracts showed DPPH radical scavenging activity in a similar manner to that of the reference antioxidant ascorbic acid- increasing activity with the increase in concentration (Fig. 3). This free radical scavenging activity might be due to the presence of phenols and flavonoids in the extracts.

Determination of Antibacterial Activity by Disc Diffusion Method: The result of antimicrobial screening of different extracts of bark and leafof *B. acuminata* have been presented in Table 4. All the ethanol extracts ofbark and leafshowedactivity against both gram positive and gram negative bacteria compared to blank. The standard &kanamycin exhibited significant zone of inhibition against all the test organisms. This antimicrobial activity is probably attributed due to the presence of saponins, flavonoids and total tannin content [23, 24] which were detected in phytochemical screening (Table 2).

CONCLUSION

The results expressed in this study are the information on the antioxidant & antimicrobial activities of leaf and bark of *B. acuminata*. The crude extracts both leaf and bark showed free radical scavenging activity when tested in different models. The crude extract was found to contain alkaloid, carbohydrate, flavonoids, saponins and tannins. The scavenging effect on DPPH and superoxide radicals represents the fraction direct radical scavenging activity. It is also seen that, the bark and leaf extracts showed zone of inhibition which indicates the antimicrobial potentiality of the plant.

ACKNOWLEDGEMENT

First of all, I would like to express my heartfelt gratitude to the Almighty Allah for being enabled me to complete such a research work. My parents also deserve special appreciation for their huge support and prayers. For that, a great thank for my family. I am privileged to express my heartfelt gratitude to my honorable senior brother, Tufikul Islam, for this expert planning, sincere direction, supervision, advices, and continuous follow up from the very beginning of this work. I would also like to acknowledge the helping hands from my friends and juniors (Samiran Kumar Sadhu, Md. Manjur Rahman & Bhuiyan Md. AshiqurRahman).

REFERENCES

1. Nasri H, (Herbal drugs and new concepts on its use). J PrevEpidemiol, 2016; 1(1): 01.
2. Butler SM (The Role of Natural Product Chemistry in Drug Discovery). J. Nat. Prod., 2004, 67 (12), 2141-2153.
3. Palhares RM, Drummond MG, Alves BS, Cosenza GP, Brandão MGL. (Medicinal Plants Recommended by the World Health Organization: DNA Barcode Identification Associated with Chemical Analyses Guarantees Their Quality). Plos One, 2015; 2.
4. Mathew S, Abraham TE. (In vitro antioxidant activity and scavenging effects of Cinnamomumverumleaf extract assayed by different methodologies). Food and Chemical Toxicology, 2006; 44: 198–206.
5. Butler MS. (The Role of Natural Product Chemistry in Drug Discovery). J. Nat. Prod., 2004; 67(12): 2141-2153.
6. Moein MR, Moein S, Ahmadizadeh S. (Radical Scavenging and Reducing Power of Salvia mirzayaniiSubfractions). Molecules, 2008; 13: 2804-2813.
7. Bibi Y, Nisa S, M Chaudhary FM, Zia M. (Antibacterial activity of some selected medicinal plants of Pakistan), BMC Complementary and Alternative Medicine, 2011; 11: 52.
8. Khan MF, Shilpi RI, Rashid RB, Rashid MA. (In vitro Antioxidant, Cytotoxic and Membrane Stabilizing Activities of Bauhinia acuminata L.). Bangladesh Pharmaceutical Journal, 2014; 17(1): 99-101.
9. Islam MN, Ferdous MR, Fahad AB, Hossain MR, Mukti M. (In-vivo Antidiarrheal and In-vitro Antimicrobial Activities of the Leaf Extracts of Bauhinia acuminata), American J. of Res. Com., 2014; 2(7): 158-168.
10. Khan MF, Shilpi RI, Rashid RB, Rashid MA. (In vitro Antioxidant, Cytotoxic and Membrane Stabilizing Activities of Bauhinia acuminata L.), Bangladesh Pharmaceutical Journal, 2014; 17(1): 99-101.
11. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. (Phytochemical screening and Extraction: A Review), Int. Pharma, 2011; 1(1): 98-106.
12. Ghani A. Medicinal Plants of Bangladesh. 2nd Ed.The Asiatic Society of Bangladesh. Dhaka, Bangladesh, 2003; 31-589.
13. Folin C, Ciocalteu V. (Tyrosine and Tryptophane Determination in Proteins – Washington), J. Biol. Chem., 1927; 73: 627-650.
14. Velioglu YS, Mazza G, Gao L and Oomah D. (Antioxidant Activity and Total Phenolics in Selected Fruits,Vegetables, and Grain Products). J. Agric. Food Chem., 1998; 46: 4113-4117.
15. Liangli Yu. (Free Radical Scavenging Properties of Conjugated Linoleic Acids). J. Agric. Food Chem., 2001; 49: 3452-3456.
16. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M and Morelli I. (Antioxidant Principles from Bauhinia tarapotensis). J. Nat. Prod., 2001; 64: 892-895.
17. Bauer AW, Kirby WMM, Sherrie JC and Tuck M. (Antibacterial Activities of Some Medicated Soaps on Selected Human Pathogens) American Journal Clinical Pathology, 1966; 451: 493-496.
18. Al-Fatimi M, Wurster M, Schroder M, Lindequist U. (Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen). Journal of Ethnopharmacology, 2007; 111: 657–666.
19. Dai J and Mumper RJ. (Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties). Molecules, 2010; 15: 7313-7352.
20. Pokorny J, Yanishlieva N and Gorodon MH. Antioxidants and food stability. In: Antioxidants in food. Cambridge CB1 6AH, England; Woodhead publishing limited, 2001; 2.
21. Kedare SB and Singh RP. (Genesis and development of DPPH method of antioxidant assay). J Food SciTechnol, 2011; 48(4): 412-422.
22. Maisuthisakul P, Suttajit M, Pongsawatmanit R. (Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants). Food Chemistry, 2007; 100: 1409–1418.
23. Avto P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z and Jurzysta M.(Antimicrobial Activity of Saponins from Medicago sp.: Structure-Activity Relationship). Phytotherapy Research, 2006; 20: 454-457.
24. Cowan MM. (Plant Products as Antimicrobial Agents). ClinMicrobiol Rev., 2002; 12: 564-582.