Research Article

www.ijrap.net

PHYTOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF METHANOLIC EXTRACT OF *ARDISIA HUMILIS* VAHL. (MYRSINACEAE)

Amina Khatun[1]*, Mahmudur Rahman[2], Sumaiya Kabir[3], Md Nahid Akter[4], Sadia Afreen Chowdhury[5]

[1]Senior Lecturer, Phytochemistry and Pharmacology Research Laboratory, Department of Pharmacy, Manarat International University, 1/B, Zoo road, Mirpur-1, Dhaka-1216, Bangladesh

[2]Mahmudur Rahman: Assistant Professor, Faculty of Health Sciences, Department of Pharmacy, Northern University Bangladesh, 24, Mirpur Road, Globe Centre, Dhaka-1205, Bangladesh

[3]Department of Pharmacy, Manarat International University, 1/B, Zoo road, Mirpur-1, Dhaka-1216, Bangladesh

[4]Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh

[5]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

Received on: 12/10/12 Revised on: 20/11/12 Accepted on: 11/12/12

***Corresponding author**
E-mail: amina_aumona@yahoo.com

DOI: 10.7897/2277-4343.04120
Published by Moksha Publishing House. Website www.mokshaph.com

ABSTRACT

Ardisia humilis Vahl. (Family-Myrsinaceae) has been traditionally used by the folklore medicinal practitioners of Bangladesh to treat cancer, heart diseases and liver poisoning where cytotoxic, thrombolytic and antioxidant medications are implicated. Besides, some other species of *Ardisia* were reported to have incriminated properties and chemical constituents. In this study, the crude methanolic extract of the *A. humilis* was evaluated for its possible cytotoxic, thrombolytic and antioxidant activities in different methods to justify some of its folklore use. Cytotoxic property of the extract was determined against brine shrimp nauplii. The thrombolytic activity was evaluated using the standard streptokinase. The antioxidant activity was measured using free radical scavenging activity with 2-2-diphenyl,1-picrylhydrazyl (DPPH) method. The extract showed significant cytotoxic effect in brine shrimp lethality bioassay where it showed the value of LC_{50} and LC_{90} 2.26 µg/ml and 7.13 µg/ml after 24 hours respectively. The standard cytotoxic drug vincristine sulphate showed LC_{50} and LC_{90} of 0.81µg/ml and 6.33µg/ml after 24 hour respectively. The study gave a significant indication of the use of the plant extract as a potential source for cytotoxic compounds. The extract showed moderate thrombolytic activity of 33.33% clot lysis where the standard streptokinase showed that of 84%. In DPPH free radical scavenging test, IC_{50} value for the methanolic crude extract was found fairly significant (4.305 µg/ml) while compared to the IC_{50} value of the reference standards ascorbic acid (2.8 µg/ml). The obtained results tend to suggest the probable cytotoxic, thrombolytic and antioxidant activities of the methanolic extract of *A. humilis* justify its use in folkloric remedies and those activities of other species of *Ardisia*.

Keywords *Ardisia humilis,* Cytotoxic, Antioxidant, DPPH free-radical scavenging, Thrombolytic, Streptokinase

INTRODUCTION

Ardisia humilis sensu F.B.I, *non* Vahl (*A. humilis*) (Family: Myrsinaceae), synonym *Ardisia solanacea* Roxb. locally known as Bara salla, Banzam, wild berry in English, is an erect, branched, glabrous shrub, under favorable conditions reaching up to 20 ft in height native to warm temperate to tropical regions of southeastern Asia like in Bangladesh, India. The plant is credited with stimulant and carminative properties[1, 2]. The plant is used in the treatment of diarrhoea, bruise, dysmenorrhoea, gout, mental disorder, rheumatic arthritis, rheumatism, skin sore and vertigo[3]. Roots are used in fever, diarrhea and rheumatism and it has antibacterial activity[4]. Other species of the *Ardisia* have been reported for their cytotoxic, thrombolytic, antitubercular and antioxidant activities[5-12]. Different constituents like beta amyrin, resorcinol analogs, ardipusilloside I, volatile oil, alkyl benzoquinones alkyl phenols and ardisiphenols have been isolated from other species of *Ardisia*[5,7,9-13].

MATERIALS AND METHODS

Collection and identification of plant material

The plant *A. humilis* was collected from Satkhira, Bangladesh in December 2011. The species was confirmed by Sarder Nasir Uddin, Principle Scientific officer, Bangladesh National Herbarium, Mirpur, Dhaka and voucher specimen of the plant has been deposited and preserved in the library of the same institution for further collection and reference (Accession number-DACB-15693).

Preparation of methanolic extract

The collected plant parts were separated from undesirable materials and then were washed with water and shade-dried for ten days. The dried plant materials were ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powdered sample was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 300 g of powered material was taken in a clean, flat-bottomed glass container and soaked in 1000 ml of methanol. The container along with its contents was sealed and kept for a period of 10 days with occasional shaking or stirring[14]. The whole mixture then underwent a coarse filtration by cotton. It was then filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate was concentrated under air and dried. It rendered a 15.4 g concentrate (5.13.0%) of greenish black color and was designated as crude methanol extract.

Test for different chemical groups

The crude methanolic extract was tested for its different chemical groups as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins using standard protocol[14-15]. 10% (w/v) solution of the extract in methanol was used for each of the above test.

Test for cytotoxic activity

The cytotoxicity assay was performed on brine shrimp nauplii using method of Mayer et al[15-16]. Brine shrimp nauplii were obtained by hatching brine shrimp eggs (Carolina Biological Supply Company, Burlington, NC, USA) in artificial sea-water (3.8% NaCl solution) for 22 hrs. Artificial sea water was prepared by dissolving 20 g of NaCl (Sodium Chloride Crystal GR, Merck Ltd., Mumbai, India) and 18 g of table salt in one liter of distilled water and was filtered off to get a clear solution. Dissolution of 30 mg of compound was performed in 3ml of artificial sea water containing 20% Dimethyl sulfoxide (DMSO, ≥99.9%, BioReagent, for molecular biology; Sigma-Aldrich, India) to give concentration of 10 µg/µl. From this solution 10, 20, 40, 80, 160 and 320 µl were transferred to each 10 ml vial and using artificial sea water volume was adjusted to 10ml by artificial sea water to give concentrations of compound of 10, 20, 40, 80, 160 and 320 µg/ml respectively. Brine shrimp nauplii were grown in these solutions and observed their mortality for 24 h. The resulting data were transformed to probit analysis software (LdP Line software, USA)[17, 18] for determination of LC_{50} values of the extract. Artificial sea-water medium containing DMSO used for the analysis was employed as negative control. Vincristine sulfate (Techno Drugs Ltd, Bangladesh) was used as standard in this assay.

***In vitro* thrombolytic activity**

5 ml of phosphate buffered saline (PBS) was added to the commercially available lyophilized streptokinase vial (15, 00,000 I.U.) and mixed properly. This suspension was used as a stock from which appropriate dilutions were made to observe the thrombolytic activity[19]. In brief, 2 ml venous blood drawn from healthy volunteers was distributed in three different pre weighed sterile microcentrifuge tube (0.5 ml/tube) and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed) and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). To each microcentrifuge tube containing pre-weighed clot, 100 µl of methanolic extract (10 mg/ml) of was added. As a positive control, 100 µl of streptokinase and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lyses. After incubation, released fluid was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lyses was expressed as percentage of clot lyses.

Test for antioxidant activity

The antioxidant activity of plant extract and the standard antioxidant were assessed on the basis of radical scavenging effect of the stable DPPH [2,2-diphenyl-1-picrylhydrazyl; Nacali tesque, Kyoto, Japan] free radical[20]. Stock solution (10 mg/mL) of the methanolic extract of *A. humilis* was prepared in respective solvent systems from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100, 500 µg/mL. In this assay, an equal amount of sample solution was added to an equal amount of 0.1 mM methanolic DPPH solution, vortex and allowed to stand at the dark place at 25°C for 30 min for the reaction to occur. After 30 min of incubation period, the absorbance was read against a blank at 517 nm with a double beam Analykjena UV/Visible spectrophotometer (Model Shimadzu, UV-1800, Japan). The radical scavenging activity was expressed as the inhibition percentage (I%) and calculated as per the equation:

$$I(\%) = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound with all reagents. IC_{50} value is the concentration of sample required to scavenge 50% DPPH free radical and was calculated from the plot of inhibition (%) against extract concentration. All the tests were carried out in triplicate and average of the absorptions was noted. Ascorbic acid was used as positive control standard for this study.

Statistical analysis

Data were presented as mean ± S.D. Statistical differences between control and treated groups were tested by Student's t-test. The differences were considered significant at $P<0.05$.

Table 1: Results of phytochemical screening of *Ardisia humilis* extracts

Phytoconstituents	Methanolic extract of *A. humilis*
Alkaloids	+
Carbohydrates	+
Flavonoids	_
Gums	
Saponins	_
Steroids	+
Tannins	+

+: Positive result; - : Negative result;

Table 2: Brine shrimp lethality bioassay of *A. humilis*

Sample	LC_{50}(µg/ml)	LC_{90}(µg/ml)	Regression equation
Methanolic extract of *A. humilis*	2.26	7.13	y=8.2143x+31.427
Vincristine sulfate	0.81	6.33	y=7.2375x+44.144

Table 3: *In vitro* thrombolytic activity of *A. humilis*

Sample	% clot lyses
Methanolic extract of A. humilis for volunteer 1	33.33 ±0.57
Methanolic extract of A. humilis for volunteer 2	33.67±1.7
Methanolic extract of A. humilis for volunteer 3	35.33±0.94
Streptokinase	85 ±1.41

Data are presented as Mean ± SD

Table 4: Antioxidant activity of *A. humilis*

Sample	IC_{50}(µg/ml)
A. humilis extract	4.305±0.275
Ascorbic acid	2.8±0.01

Data are presented as Mean ± SD

RESULTS

Chemical group test

Results of different chemical tests on the methanolic extract of *Ardisia humilis* showed the presence of alkaloids, carbohydrates, steroids and tannins (Table 1).

Cytotoxic activity

Table 2 shows the cytotoxic effect of the methanolic extract of *A. humilis* using brine shrimp lethality bioassay. In the test, the extract showed LC_{50} and LC_{90} of 2.26 µg/ml and 7.13µg/ml after 24 hours respectively. Where, standard vincristine sulphate showed LC_{50} and LC_{90} of 0.81µg/ml and 6.33µg/ml after 24 hour respectively. No mortality was found in the control group. An approximate linear correlation was observed when concentrations versus percentages of mortality were plotted on graph paper.

Thrombolytic activity

Table 3 shows the effect of the methanolic extract on clot lyses activity. The percentage (%) clot lysis was statistically significant ($p<0.001$) when compared with vehicle control. The plant extract showed moderate colt lyses activity (33.33±0.57%, 33.67±1.7% and 35.33±0.94% for volunteer 1, 2 and 3 respectively whereas standard streptokinase showed 85±1.41% clot lyses activity).

Antioxidant activity

Free radical scavenging activities of the methanolic extract of *A. humilis*, measured by DPPH assay, are shown in Table 4. DPPH free radical scavenging capacity was found to be increased with the increase of concentration of the extract. The extract showed significant antioxidant activity where the IC_{50} 4.305±0.275 µg/ml compared with the standard ascorbic acid that showed IC50 at 2.8±0.01 µg/ml.

DISCUSSION

The methanolic extract of *Ardisia humilis* demonstrated the presence of alkaloids, carbohydrates, steroids and tannins as secondary metabolites with potential biological activities. Several species of *Ardisia* were reported for free phenolic content, beta amyrin type pentacyclic triterpenoid compounds and antioxidant activities. Presence of these compounds and activities may be due to the presence of tannins and other phenolic compounds[9, 12]. Further bioassay guided approaches may be taken to elucidate the responsible compounds[21].

Brine shrimp lethality bioassay is an easy and straight forward bench top screening method for predicting important pharmacological activities like enzyme inhibition, ion channel interference, antimicrobial and cytotoxic activity[16, 22]. Several species of *Ardisia* were reported for suppressor of skin tumor, inhibitor of tumor induced angiogenesis and cytotoxic activities[5-8, 11]. In the present study the extract showed LC_{50} at a low concentration indicating that the extract is significantly potent. Ideally, any agent useful in the treatment of cancer should not be toxic to normal cell. However, in reality, anticancer agents are often toxic to normal cells, particularly towards rapidly growing cells[23]. It is necessary to test this extract against various cancer cell lines as well as normal cell lines to justify the potential to further investigate this plant for anticancer activity. Further investigation is required to find the responsible compound(s) for the cytotoxic activity observed for *Ardisia humilis*.

The thrombolytic bioassay result suggested that the extract may contain pentacyclic triterpenoid compounds that possess important antithrombotic active constituents and provided information for the exploitation and utilization of triterpenoid compounds as thrombin inhibitors for the treatment of thrombotic disease[9].

Antioxidant activity of plants is one of the great virtues which can be exploited to prevent various diseases including cancer. Daily ingestion of antioxidant constituents with our diet can significantly reduce the chance of cancer by fighting against free radicals[24, 25]. In the present study the extract showed significant antioxidant activity and was comparable to that of ascorbic acid, used as the positive control in this assay. Antioxidant compounds cannot be treated as therapeutic agent for cancer treatment since antioxidants prevent the development of cancer through a protective manner while anticancer drugs directly affects the cellular function of cancerous cell[26]. However, there are reports that antioxidants like tea phenolics can directly inhibit cancer cells[27].

Present study is based on the report of preliminary phytochemical and biological screening of *A. humilis* extract. The results are quite promising; support the use of this plant in traditional medicine and demand further investigation. Advanced studies including LC-MS can be carried out to get a bigger picture of the chemical constituents present in the plant. Screening methods applying various cell lines or bacterial enzymes can be carried out to find the underlying mechanism for the observed biological activities. On the basis of the results from above studies, bioassay guided approach can be undertaken to isolate and identify the active

component(s).

ACKNOWLEDGEMENTS

We are grateful to the authority of Bangladesh National Herbarium, Dhaka, Bangladesh for its support by identification, providing library and plant database resources and overall inspirations during this research work. We thank the Head of the Departments, Department of Pharmacy, Manarat International University and Northern University Bangladesh for allowing us to use their lab facilities.

REFERENCES

1. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Allahabad: International Book Distributors; 2006, 2:1483–4.
2. Ahmed N. Wild Flowers of Bangladesh. Dhaka: The University Printing Press; 2007, 60.
3. Uddin NS. Traditional Uses of Ethnomedicinal Plants of the Chittagong Hill Tracts. Dhaka: Bangladesh National Herbarium; 2006, 370.
4. Ghani A. Medicinal Plants of Bangladesh. 2nd ed. Dhaka: The Asiatic Society of Bangladesh; 2003,105.
5. Chen LP, Zhao F, Wang Y, Zhao LL, Li QP, Liu HW. Antitumor effect of resorcinol derivatives from the roots of Ardisia brevicaulis by inducing apoptosis. J Asian Nat Prod Res 2011; 13(8):734-43. http://dx.doi.org/10.1080/10286020.2011.587412 PMid:21751842
6. Roslida A, Fezah O, Yeong LT. Suppression of DMBA/croton oil-induced mouse skin tumor promotion by Ardisia Crispa root hexane extract. Asian Pac J Cancer Prev 2011; 12(3):665-9. PMid:21627361
7. Wang R, Gu Y, Zhang WD, Yan XN, Jin L, Wang XJ. Inhibition of tumor-induced angiogenesis and its mechanism by ardipusilloside I purified from Ardisia pusilla. J Asian Nat Prod Res 2012; 14(1):55-63. http://dx.doi.org/10.1080/10286020.2011.631182 PMid:22263594
8. Wang X, Tang S, Zhai H, Duan H. Studies on anti-tumor metastatic constituents from Ardisia Crenata. Zhongguo Zhong Yao Za Zhi 2011; 36(7):881-5. PMid:21761727
9. Ching J, Chua TK, Chin LC, Lau AJ, Pang YK, Jaya JM, et al. Beta-amyrin from Ardisia elliptica Thunb. is more potent than aspirin in inhibiting collagen-induced platelet aggregation. Indian J Exp Biol 2010; 48(3):275-9. PMid:21046981
10. Chang CP, Chang HS, Peng CF, Lee SJ, Chen IS. Antitubercular resorcinol analogs and benzenoid C-glucoside from the roots of Ardisia cornudentata. Planta Med 2011; 77(1):60-5. http://dx.doi.org/10.1055/s-0030-1250094 PMid:20607650
11. Chang HS, Lin YJ, Lee SJ, Yang CW, Lin WY, Tsai IL, et al. Cytotoxic alkyl benzoquinones and alkyl phenols from Ardisia virens. Phytochemistry 2009; 70(17-18): 2064-71. http://dx.doi.org/10.1016/j.phytochem.2009.09.006 PMid:19833361
12. Sumino M, Sekine T, Ruangrungsi N, Igarashi K, Ikegami F. Ardisiphenols and other antioxidant principles from the fruits of Ardisia colorata. Chem Pharm Bull 2002; 50(11):1484-7. http://dx.doi.org/10.1248/cpb.50.1484
13. Pu LX, Yuan XH, Tang TJ. Analysis of volatile oil from Ardisia brevicaulis. Zhong Yao Cai 2009; 32(11):1694-7. PMid:20218292
14. Nayeem AA, Khatun A, Rahman MS, Rahman M. Evaluation of phytochemical and pharmacological properties of Mikania cordata (Asteraceae) leaves. J of Pharmacognosy and Phytotherapy 2011; 3(8): 111-23.
15. Rahman M, Khatun A, Rahman SM, Rashid MA. Antioxidant, Antimicrobial and Cytotoxic activities of Vitis trifolia Linn. J of Dhaka Int Univ 2010; 1(1): 181-4.
16. Meyer BN, Ferrigni NR, Putnam JB, Jacobsen LB, Nichols DE, JL M. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 1982; 45:31-4. http://dx.doi.org/10.1055/s-2007-971236
17. Uddin SJ, Grice ID, Tiralongo E. Cytotoxic Effects of Bangladeshi Medicinal Plant Extracts. Evid Based Complement Alternat Med 2011; 1-6. http://dx.doi.org/10.1093/ecam/nep111 PMid:19706693 PMCid:3144735
18. Finney DJ. Probit analysis. 3rd ed. Cambridge: University Press; 1971, 18, 37-77.
19. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. Thromb J 2006; 4(14): 1-4.
20. Gupta M, Mazumder UK, Sivahkumar T, Vamis MlM, Karki S, Sambathkumar R, et al. Antioxidant and antiinflammatory activities of Acalypha fruticosa. Nig J Prod Med 2003; 7:25–9.
21. Ahmed F, Ohtsuki T, Rahman M, Sadhu SK, Toume K, Ishibashi M. Cryptolepine, isolated from Sida acuta, sensitizes human gastric adenocarcinoma cells to TRAIL-induced apoptosis. Phytotherapy Research 2010; 25(1):147–50. http://dx.doi.org/10.1002/ptr.3219 PMid:20623717
22. Anderson JE, Goetz CM, McLaughlin JL, Suffness M. A blind comparison of simple bench-top bioassay and human tumour cell cytotoxicities as antitumor prescreens. Phytochem Analysis 1991; 2:107-11. http://dx.doi.org/10.1002/pca.2800020303
23. Priestman, T. Cancer Chemotherapy in Clinical Practice. London: Springer-Verlag; 2008, 130-6.
24. Soler-Rivas C, Espín JC, Wichers HJ. An easy and fast test to compare total free radical scavenger capacity of foodstuffs. Phytochem Anal 2000; 11:330-8. http://dx.doi.org/10.1002/1099-1565(200009/10)11:5<330::AID-PCA534>3.0.CO;2-G
25. Naik GH, Priyadarsini KI, Satav JG. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochemistry 2003; 63:97-104. http://dx.doi.org/10.1016/S0031-9422(02)00754-9
26. Ewan S. Cancer prevention a step nearer. Drug Discov Today 2005; 10:950-1. http://dx.doi.org/10.1016/S1359-6446(05)03527-0
27. Senior K. Tea: a rich brew of anti-cancer magic bullets? Drug Discov Today 2001; 6:1079-80. http://dx.doi.org/10.1016/S1359-6446(01)02022-0

Cite this article as:

Amina Khatun, Mahmudur Rahman, Sumaiya Kabir, Md Nahid Akter, Sadia Afreen Chowdhury. Phytochemical and pharmacological properties of methanolic extract of Ardisia humilis vahl. (Myrsinaceae). Int. J. Res. Ayur. Pharm. 2013; 4(1):38-41

Source of support: Nil, Conflict of interest: None Declared