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Anti-microbial screening and cytotoxic activity: *In-vitro* analysis of *Persicaria glabra*

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Abstract
Medicinal plants are the key source of medicines in human life. This present study was aimed to evaluate the anti-microbial and cytotoxic activity of the methanolic extract of *Persicaria glabra* whole plant. The pharmacognostic analysis of *Persicaria glabra* has been useful to identify authentic compounds and recognize isolates. The methanolic whole plant extract of *Persicaria glabra* showed antimicrobial activity against different bacterial strains, including gram negative, gram positive, and three fungal strains using the agar diffusion method. The methanolic extract showed broad spectrum antimicrobial activity *Aspergillus niger*. Methanol extract showed high cytotoxicity value with low IC50 values. Antimicrobial studies have shown that the whole plant of *Persicaria glabra* has antimicrobial activity against selected pathogens. The brine shrimp lethality test is an excellent predictive tool for the toxic potential of plant extracts in humans.

Keywords: Anti- microbial activity, cytotoxic activity, methanolic extract, *Persicaria glabra*

Introduction
At the beginning of modern era, medicinal plants are playing an important part for human to prevent development of various diseases. Traditional medicines use parts like leafs, roots and barks to treat different diseases. 87% of drugs were came from natural source and about 25% of used drugs made from plants; 80% people depends on these medicine for their betterment [9]. The amount of higher plants mainly angiosperms and gymnosperms on earth are 250,000 [5], with an upper level as high as 500,000 [11, 4]. Among them around 6% have been analyzed for biological activities. Whereas, phytochemical investigations have been run on 15% plants [12]. Approximately, 60% of pharmaceuticals are from plant origin and the use of expensive intermediary chemicals will also fell down if people starting rely on plant derivatives [2]. Cosmetics, detergents, dyes, insecticides, foods and paints manufacturer also using plants [19] .These medicinal plants have active compounds which provide antiviral, antibacterial, antifungal, anti-inflammatory, anti-helminthic and antioxidant activity [10]. In addition, Bangladesh is enriched with more than 5,700 angiosperm species, 1,700 species of pteridophytes and 3 species of gymnosperms, among which a total of 24 plants are in various degrees of the threat of extinction [1 3].

The medicinal plant *Persicaria glabra* belongs to Polygonaceae family. Locally it is known as Bihagni or Lal Kukri, and the common name of this plant is dense flower knotweed, smooth smartweed. This is an entirely glabrous plant, except the leaves which are often red-gland dotted, ochrea is completely eciliate. It has red small flowers. Grows mostly from plains to 1,000 m in or near water, in ditches, river banks, margins of lakes, pools and wadis, where it often forms dense, mono-specific stands.

Polygonaceae family provided us with therapeutic uses such as asthma, bronchitis, cough, diarrhea, dysentery, eczema, earache, inflammatory conditions, jaundice, kidney disease, leprosy, paralysis, toothache, ulcerative colitis, intestinal parasites and others. The species Persicaria also provided medicinal property to treat colic pain, skin conditions such as scabies, boils, abscesses and ringworms. Persicaria species has phytochemical compounds included flavonoids, terpenoids, anthraquinones and apianen lactones which revealed anticancer, antioxidant, analgesic, antileukemic, antimicrobial and tyrosinase inhibiting properties [8, 14]. Plants of this Polygonaceae family contained many pharmacological activities like *Persicaria*

barbata (L) seeds has antiemetic, purgative, stimulant properties and can be used torelieve colic pain. It has anthelmintic and diuretic activities too [15]. Another plant *Persicaria orientalis* has anti-ulcer properties [16, 13]. *Persicaria hydropiper* plant has antioxidant, antipyretic, astringent, laxative, styptic and antibacterial properties. This plant also has antitumor, antifungal and antiproliferative properties [17, 18]. In addition, A pure anthelmintic and molluscicidal terpenoid substance (PGA) has been derived from the methanolic extract of the leaves [19]. So, this plant *Persicaria glabra* can be a potential source of medicinal properties and for this purpose this study was designed to find out cytotoxic and antimicrobial activity.

Materials and Methods

Plant collection and identification

The whole plant of *Persicaria glabra* was collected in the month of October 2016 from Khagrachari, Bangladesh. After that, its verification was done by the National Herbarium of Bangladesh (NHB), Mirpur, and Dhaka by submitting plant sample. Verification code number was 45023.

Preparation of plant extract

Leaves were shade dried for several days after proper washed with water. After that leaves were grounded finely as a granular particle with a high power grinding machine. About 400gm of grounded leave powder of *Persicaria glabra* was obtained. Then these powder was soaked in 2L of methanol for 14 days period in a room temperature (22-25ºC) with random stirring. After 14 days of soaking, the mixture was filtered by using Watmann filter paper (pore size 100nm). The filtrate was concentrated by using rotary evaporator (Heidolph) at 30ºC temperature with a rotation speed of 100rpm up to form the concentrated methanolic extract.

Chemicals and Drugs

Methanol collected from Active Fine Chemicals Limited., Bangladesh. Vincristine sulphate was obtained from Beacon Pharmaceuticals Limited, Bangladesh and Dimethyl sulfoxide (DMSO) was purchased from Fisher Scientific, UK. Nutrient Agar, Nutrient Broth, M. H. Agar was obtained from Becton Dickinson GmbH, Germany and Kanamycin was acquired from Beximco Pharmaceuticals Limited, Bangladesh.

Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay is used to identify cytotoxicity [20] and it also help to find other important pharmacological activities such as antimicrobials, antivirals, pesticides and tumor-resistant, etc. mixtures. [21]. In this study, One liter water taken into a small tank and 38 g of sea salt were dissolved in it. Then it was filtered to obtained clear solution. *Artemia salina* was incubated and developed as nauplii in the tank. 10 live nauplii were transferred into each of the test tubes containing 5 ml of seawater. Test sample was removed from vial and added in 100 µl of dimethylsulfoxide to obtain stock preparations. After that, test sample added in to the test tubes. The concentration was prepared in the first test tube was 400 µg / ml. By the help of serial dilation process different concentration obtained. Here, 50 µl of test sample were added to the test tube and 50 µl of new DMSO were added to the vial.

Antimicrobial screening:

Here, Disc diffusion method were used to determine antimicrobial activity by measuring zone of inhibition. In patridishes restricted source through nutrient agar gel used and sterilized filter paper discs (6mm in diameter) contained the test sample in a specific amount which is positioned on nutrient agar medium with microorganisms. As a standard of antibiotic, kanamycin discs and blank discs are used as a positive and negative control. Those plates are kept at 4°C temperature for 24 hours to allow maximum diffusion of the test materials to the surrounding media [6]. After 24 hours those plates are inverted and incubated at 37°C for 24 hours for optimal growth of microorganisms. If the test materials have antimicrobial property, it will inhibit the growth of microorganisms in the near to the discs of that media and produce a clear distinct area which defined as a zone of inhibition. After that, the antimicrobial activity of test agent is then measured by determining the diameter of the zone of inhibition in millimeter [6 7].

Collection of bacterial strains

Different gram positive and gram negative bacterial and fungal stains were collected from the microbiology lab of the department of Pharmacy, University of Dhaka.

Table 1: Different strains used in antimicrobial screening

Gram negative Bacteria	Gram positive Bacteria	Fungi
Escherichia coli Salmonella paratyphi Salmonella typhi Shigella boydii Shigella dysenteriae Pseudomonas aeruginosa Vibrio mimicus Vibrio parahemolyticus	Bacillus cereus Bacillus megaterium Bacillus subtilis Sarcina lutea Staphylococcus aureus	Aspergillus niger Candida albicans Sacharomyces cerevacae

Sterilization Procedure

The antimicrobial screening process was run under the laminar airflow cabinet to avoid any type of cross contamination. Moreover, The autoclaving was done at a 121ºC temperature and maintaining 15lbs/square pressure to sterile the petri dishes and glass instruments. Laminar hood, cotton, blank discs, micropipette tips and forceps were sterilized by keeping under UV light for one hour.

Preparation of Subculture

Test organisms transferred from stock culture to agar plates under an aseptic condition in the laminar air cabinet. After that those inoculated strains were incubated at 34ºC temperature for 24 hours to gain optimum growth of those microorganisms.

Preparation of the test plate

Under the aseptic area, a subculture of test organisms was moved into the test tube which contains around 10ml of sterilized and melted agar medium with the support of a sterilized transfer loop. Those test tubes were shaken with vortex machine to achieve an even distribution of microorganism suspension. After that, suspension of microorganisms (bacteria and fungus) were transferred in the sterile Petri dish. Several times rotation of Petri dishes as clockwise and anticlockwise was done so that uniform distribution of test microorganisms occur in media.

Preparation of Discs

The calculated amount of test sample was dissolved in methanol to get the desired concentrations in a sterilized

condition. After that, the discs were soaked with solutions of test samples and dried. The concentration was 400 µg/disc. As a positive control standard Kanamycin (30 µg /disc) plates were used to assure the activity of standard antibiotic against the test organisms. Blank discs were used as negative controls to confirm that the filter paper and the solvents were not contaminated.

Diffusion and Incubation

Our sample discs, control discs and standard antibiotic disc were placed smoothly on the marked zone of agar plates which were pre-inoculated with test bacteria and fungus. After that, those plates were putted in a cool place for 24 hours at a 4°C temperature. After that those plates were incubated for another 24 hours at 37°C temperature.

Determination of the zone of inhibition

The zone of inhibition measured by a transparent scale to determine the potency of test material as an antimicrobial agent. The diameter of the zone of inhibition in millimeter unit indicates the prevention of growth of microorganisms by test material.

Results and Discussion

Brine shrimp lethality bioassay

The methanolic extract (ME) of leaves of *Persicaria glabra* was examined for brine shrimp lethality bioassay. The cytotoxicity of the extract to brine shrimp was observed and the results are given in Table 2.

The lethal concentration (LC_{50}) of the test sample was determined by plotting the percentage of the mortality rate of shrimps against the logarithm of concentration. The curve of regression analysis helps in gaining the best-fit line (Figure 1 & 2). Vincristine sulfate (VS) was used as positive control and the LC_{50} was found to be 0.35 µg/ml. The LC_{50} of the methanolic extract of leaves of *Persicaria glabra* was 0.516 µg/ml which is much higher than vincristine sulfate. From this analysis it can be say that this plant cytotoxic activity and it can be used as cytotoxic agent after isolation of responsible compound.

Table 2: LC_{50} values of the test samples of leaves of *Persicaria glabra* and standard vincristine sulphate**.**

Samples	LC_{50} (µg/ml)
VS	0.35
ME	0.516

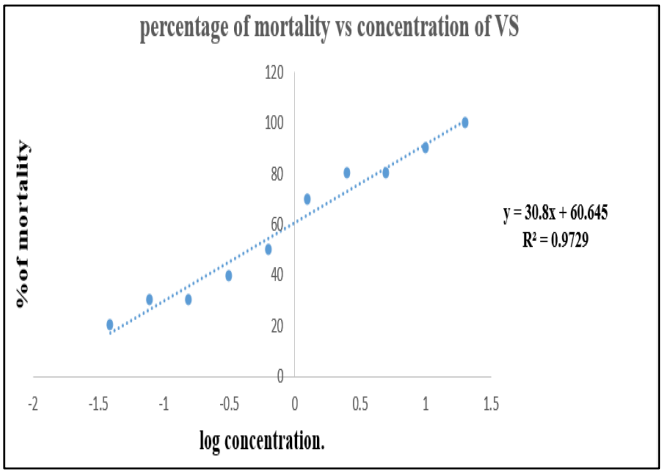

Fig 1: Plot of % of mortality and predicted regression line of VS

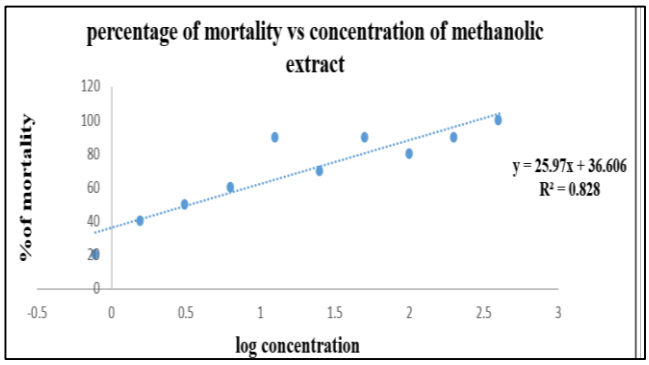

Fig: Plot of % mortality and predicted regression line of ME

Antimicrobial Screening

The methanolic extract (ME) of leaves of *Persicaria glabra* was investigated to find out the antimicrobial activity with a concentration of 400 µg/disc in every case. The methanolic extracts of the leaves of *Persicaria glabra* showed some moderate antimicrobial activity against some different tested microorganisms (Table 3).

The maximum zone of inhibition exhibited by ME was found to be 11mm against *Aspergillus niger*. The mild antimicrobial activities were tested against *Sacharomyces cerevacae* (having zone of inhibition of 10 mm), *Escherichia coli* (having zone of inhibition of 9 mm), *Sarcina lutea* (having zone of inhibition of 9 mm), *Bacillus subtilis* (having zone of inhibition of 8 mm), *Vibrio parahemolyticus* (having zone of inhibition of 18 mm), *Bacillus cereus* (having zone of inhibition of 7 mm), *Vibrio mimicus* (having zone of inhibition of 5 mm), *Bacillus megaterium* (having zone of inhibition of 4 mm), *Staphylococcus aureus* (having zone of inhibition of 4 mm), *Shigella boydii* (having zone of inhibition of 3 mm). Among all of the test microorganisms, the lowest activity was exhibited against *Pseudomonas aeruginosa* (having a zone of inhibition of 2 mm).

Table 3: Antimicrobial activity of test samples of bark of *Persicaria glabra*

Test microorganisms	Diameter of zone of inhibition (mm)	
	Extract	Kanamycin
Gram Positive Bacteria		
Bacillus cereus	7	37.6
Bacillus megaterium	4	38.3
Bacillus subtilis	8	35.0
Staphylococcus aureus	4	35.0
Sarcina lutea	9	37.3
Gram-negative bacteria		
Escherichia coli	9	37.0
Pseudomonas aeruginosa	2	35.6
Shigella boydii	3	35.1
Vibrio mimicus	5	37.3
Vibrio parahemolyticus	8	38.0
Fungi		
Aspergillus niger	11	37.0
Sacharomyces cerevacae	10	38.6

Conclusion

In this study, Brine Shrimp Lethality Bioassay indicated the presence cytotoxic activity in the plant *Persicaria glabra*. The methanolic extract of plant showed singnificant level of result. The disc diffusion method revealed moderate level antimicrobial activity. Where methanolic extract showed activity against all the bacteria and fungi. Further investigation can be conducted with different fractions and

isolation of compounds for comprehensive information.

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Reference

1. Zulfiker HM, Zulfiker AHM, Roy PP, Momin MAM, MS Khan. Investigation of Antioxidant and Antimicrobial Potential of Chloroform and Petroleum Ether Extracts of Selected Medicinal Plants of Bangladesh. British Journal of Medicine & Medical Research. 2013; 3(4):418-1436.
2. Ali M. Textbook of Pharmacognosy. 2nd edition. CBS publishers and distributors. 2010, 20.
3. Ali Reza AHM, Barua Shuvashish P, Huq SM, Imamul Khan AS, Moniruzzaman, Nishat Ainun. Bio-ecological zones of Bangladesh. IUCN Publication ISBN: 2002; 984(31):1090-0.
4. Ayafor JF. Limonoids and phytol derivatives from Cedrela sinensis. Tetrahedron. 1972, 28, 9343.
5. Ayensu ES, DeFilipps RA. Endangered and Threatened Plants of the United States. Washington, DC: Smithsonian Institution. 1978.
6. Barry AL. Principle and practice of Microbiology. 3rd Ed., Lea and Fabager,Philadelphia. 1976
7. Bayer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. 1966; 45:493-496.
8. Bozin B, Mimica-Dukic N, Samojlik I, Goran A, Igic R. Phenolics as antioxidants in garlic (Allium sativum L., Alliaceae). Food Chem. 2008; 111:925-929.
9. Khatun A, Rahman M, Haque T, Rahman MdM, Akter M, Akter S *et al*. Cytotoxicity Potentials of Eleven Bangladeshi Medicinal Plants. The Scientific World Journal, 2014, 1-7. doi:http://dx.doi.org/10.1155/2014/913127.
10. Latifou L, Eugenie A, Menonve A, Brice A, Karim D, Ambaliou S. Antimicrobial activity of Crataeva religiosa Frost against bacteria isolated from Thryonomys swinderianus Remminck. African Journal of Biotechnology, 2011; 10(49), 10034-10039. ISSN 1684-5315.
11. Tippo O, Stern WL..Humanistic Botany. New York:W.W. Norton. 1977.
12. Verpoorte R. Pharmacognosy in the new millennium: lead finding and biotechnology. J Pharm Pharmacol 2000; 52:253–262
13. Kamal Uddin, Mahbubur Rahman AHM, Rafiul Islam AKM. Taxonomy and Traditional Medicine Practices of Polygonaceae (Smartweed) Family at Rajshahi, Bangladesh, International Journal of Advanced Research. 2014; 2(11):459-469.
14. Khatun. Antinociceptive effect of methanol extract of leaves of Persicaria hydropiper in mice, BMC Complementary and Alternative Medicine, 2015; 15:63
15. Ahmed F, Rahman S, Ahmed N, Hossain M, Biswas A, Sarkar S. Evaluation of neolamarckia cadamba (roxb.) Bosser leaf extract on glucose tolerance in glucose-induced hyperglycemic mice. Tradit Complement Altern Med. 2011; 8(1):79-81
16. Keya MA, Rahman AHMM. Angiosperm Diversity at the Village Sabgram of Bogra, Bangladesh with Emphasis on Medicinal Plants. American Journal of Plant Biology. 2017; 2(1):25-34.
17. Huq AKMM, Jamal JA, Stanslas J. Ethnobotanical, Phytochemical, Pharmacological, and Toxicological Aspects of Persicaria hydropiper (L.) Delarbre. PMC. US National Libraryof Medicine National Institutes of Health. Published online, 2014, 16.
18. Lajter I, Zupkó I, Molnár J, Jakab G, Balogh L, Vasas A. Antiproliferative Activity of Polygonaceae Species from the Carpathian Basin against Human Cancer Cell Lines. Phytother. Res. published online 4 April 2012 in Wiley Online Library. 2013; 27:77-85.
19. Peace N, Eneh, Corey L, Horien Warda Ashraf, Amanda J. Bakken1 and Jennifer L. Bath A randomized survey of medicinal plants used as. Journal of Medicinal Plants Research. 2012; 7(9):543-550. doi:DOI:10.5897/JMPR012.1132.
20. Meyer B, Ferrigni N, Putnam J, Jacobsen L, Nichols D, &amp. McLaughlin, J. Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. Planta Medica, 1982; 45(5):31-34.https://doi.org/10.1055/s-2007- 971236
21. McLaughlin JL, Rogers LL, &amp. Anderson, JE The Use of Biological Assays to evaluate Botanicals. Drug Information Journal, 1998; 32(2):513-524. https://doi.org/10.1177/009286159803200223