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Bioactivity evaluation of commercial root samples of *Hemidesmus indicus* (L.) R.Br.

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Abstract

Four commercial root samples (CS1, CS2, CS3 and CS4) of *Hemidesmus indicus* alongside an authentic sample (HI) were evaluated following antibacterial potential, cytototoxicity and DPPH free radical scavenging activity assay. The inhibition zones produced by commercial samples ranged between 9.5 to 13.5 mm against the tested bacteria in antibacterial sensitivity test. HI produced highest inhibition zone recorded as 20.63 mm against *Staphylococcus aureus* followed by 17.4 mm against *Bacillus subtilis* at 150 µg/ml concentration**.** HI also showed highest cytotoxicity with LC_{50} value of 1.726 mg/ml, 4.754 mg/ml and 13.247 mg/ml after 6 hours, 12 hours and 24 hours respectively in brine shrimp lethality assay. Sample CS1 and CS2 showed less cytotoxicity compared to CS3 and CS4. Depending on the concentration of extracts, the DPPH free radical scavenging activities of commercial samples were in close proximity with HI. The total phenolic content in HI was 54.52 mg/ml while in four commercial sample, it ranged between 52.28 to 75.37 mg/ml gallic acid equivalent (GAE) per 100 mg extract.

Key words: *Hemidesmus indicus*, Antibacterial assay, Cytotoxicity, Antioxidants.

INTRODUCTION

Plants are source of many important medicines since ancient time. A large number of medicinal plants are now widely used all over the world for production of both traditional and modern drugs. *Hemidesmus indicus* (L.) R.Br. (family: Apocynaceae), locally known as Anantamul, is a medicinal plant being traditionally used for the treatment of various diseases and aliments in Ayurvedic and Unani systems of medicine (Lalrinpuia *et al*., 2017). It is a twining shrubby climber with small opposite lanceolate sessile leaves, small flowers in axillary cluster and tortuous stout long roots, grows in different areas of Bangladesh (Ghani, 2003). *H. indicus* has been reported to have anti-inflammatory, antioxidant, antipyretic, antimicrobial and anti-leprotic properties (Chopra *et al.*, 1956). It is used as blood purifier and has diuretic, antirheumatic, antidiarrhoeal and anti-viper venom activity (Bell *et al*., 1980). Its' roots serves as remedy for leprosy, syphilis, leucoderma, asthma, dysentery, fever as well as blood, kidney and urinary diseases (Shete & Bodhankar, 2010).

From the pharmacological point of view, *H. indicus* has been studied for the first time in 1962, when the diuretic potential of its roots has been explored (Satoskar *et al*., 1962). Since then, a number of reviews and numerous other specific articles on the

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pharmacology of *H. indicus* have been published (Austin, 2008; Aneja *et al*., 2008; George *et al*., 2008; Das & Bisht, 2013), suggesting a wide range of beneficial effects. Herbal drug manufacturer in Bangladesh use *H. indicus* in a regular basis for manufacturing a number of useful drugs. But safety of plant-based medicines has frequently been questioned due to reported toxic effects and hence safety evaluation is important for hazard identification and standardization of novel drugs (De Smet, 1995). Due to the increasing demand for roots of this species, there is a known tendency for deliberate adulteration by other plants (Sharma *et al.*, 2002). Austin, (2008) reported that this pharmacologically important plant possesses a number of potent bioactive compounds. There are a number of reports on bioactivity evaluation of *H. indicus* root extract from around the world (Das & Devaraj, 2006; Kavita *et al*., 2010; Shete & Bodhankar, 2010; Saha *et al*., 2014; Lalrinpuia *et al*., 2017). But there is lacking information on bioactivity based evaluation of its' commercial samples in Bangladesh. Thus, bioactivity assays namely, antibacterial sensitivity, brine shrimp lethality and DPPH free radical scavenging activity were carried out for four commercial root samples and compared to an authenticated root sample of *H. indicus* collected from Jahangirnagar University campus, Savar, Dhaka.

MATERIALS AND METHODS

Commercial root samples of *H. indicus* were collected from Moulvibazar district in Sylhet Division, wholesalers of Puran Dhaka and Namabazar, Savar, Dhaka and labeled as CS1, CS2, CS3 and CS4. Besides, an authentic root sample of *H. indicus*, collected from Jahangirnagar University campus, was brought to the Plant Taxonomy Laboratory, Department of Botany, Jahangirnagar University for authentication and identified through observing the morphological characters. After proper identification, the plant sample was labeled as HI and a voucher herbarium specimens bearing the accession no. DACB 45702 was deposited in Bangladesh National Herbarium, Mirpur, Dhaka.

The collected samples were dried separately under open sunlight followed by drying in a hot air oven (Gallenkamp) at moderate temperature (not more than 50 °C) for 96 hours to make it suitable for grinding. The dried samples were ground into coarse powder using a high capacity grinding mill and stored in air-tight container in a cool, dark and dry place for further investigation. 100 g of each of the powdered samples were digested in 250 ml of 100% methanol for three days accompanying with occasional shaking and stirring. The extracts were filtrated by clean, white cotton material followed by further filtration with Whatman no. 1 filter paper. Digested residual samples were re-digested with fresh methanol and the whole process was repeated three times for each of the six samples to obtain maximum amount of extract. The extracts were concentrated at 45 °C under reduced pressure using a rotary evaporator and resultant residues were stored under refrigerated conditions until further studies.

Antibacterial sensitivity test was estimated by the disc diffusion method following Bauer *et al*., (1966). The bacterial strains used for the experiment were collected as pure cultures from Microbiology Laboratory, Department of Botany, Jahangirnagar University, Savar,

Dhaka. Gram-negative (*E. coli*, local isolate) and Gram-positive (*Bacillus subtilis*, *Streptococcus* sp., and *Staphylococcus aureus*) bacteria were taken for the test. The sample discs, the standard antibiotic disc and the control disc were placed gently on the previously marked zones in the nutrient agar plates. The plates were then inverted and kept in an incubator at 37 °C for 24 hours. After incubation, the antimicrobial activities of the bacteria were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

The cytotoxicity assay was carried out following Brine shrimps *(Artemia salina*) lethality bioassay as described by Mayer *et al*., (1982). Antioxidant activities of the extracts were measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity following the method described by Blois (1958) and Aoshima *et al*. (2004). The absorbance of each of the sample along with the control were measured at 517 nm using a UV/VIS Spectrophotometer (PD-303S). The scavenging activity was calculated using the following formula:

Scavenging (%) = $(A_{control} - A_{sample})/A_{control} \times 100$, where A_{sample} is the absorbance of the test sample and $A_{control}$ is the absorbance of the control.

The amount of total phenols present in the root extract was determined using Folin-Ciocalteu (FC) reagent by a formerly reported method of Song *et al*., (2010). The phenolic content was evaluated from Gallic acid standard curve and measured as mg/ml GAE per 100 mg extract.

RESULTS AND DISCUSSION

Antibacterial effect of methanolic crude extract of *H. indicus* and the four commercial root samples were tested against *Bacillus subtilis, Streptococcus sp., Staphylococcus aureus* and *E. coli.* Crude methanolic extract of HI exhibited highest antibacterial activity by producing 20.63 mm inhibition zone against *Staphylococcus aureus* followed by 17.4 mm against *Bacillus subtilis* at 150 µg/ml concentration (Fig. 1). Among the four commercial samples, antibacterial potentiality of CS1 and CS3 were relatively higher while CS4 showed the lowest inhibition values against the tested bacteria. CS3 showed highest activity against *E. coli* by producing 17.13 mm inhibition zone at 150 µg/ml concentration followed by 16.63 mm at 100 µg/ml concentration. *Streptococcus* sp. showed minimum response to the extracts as the values of inhibition zones varied between 8.25 to 13.12 mm. Antibacterial potential of genuine *H. indicus* (HI) was highest compared to the commercial samples (Fig. 1).

The chloroform extract of roots of *H. indicus* have been reported to possess antibacterial effect against *Helicobacter pylori* from humans (Austin *et al*., 2003). Antidiarrhoeal effect of methanol extract of *H. indicus* against *Salmonella typhimurium, Escherichia coli* and *Shigella flexneri* have been reported in experimentally-induced diarrhea in rats (Das & Devaraj, 2006). Gayathri & Kannabiran (2009) studied the antibacterial activity of *H. indicus* and showed significant inhibition zone of 14 ± 0.01 mm against *Staphylococcus*

aureus. The root extracts of *H. indicus* in different ratios of Petroleum ether and Ethyl acetate, have shown significant zone of inhibition to *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginos*a (Kavita *et al*., 2010). Joseph *et al*., (2011) studied the antibacterial activity of different extract of *H. indicus* against 12 human pathogenic bacteria and found that methanolic extract had significant inhibitory effect against *E. coli*, *Staphylococcus aureus* and *Streptococcus faecalis*. However, above mentioned reports showed a clear consistency with the present findings of the antibacterial activities of *H. indicus.*

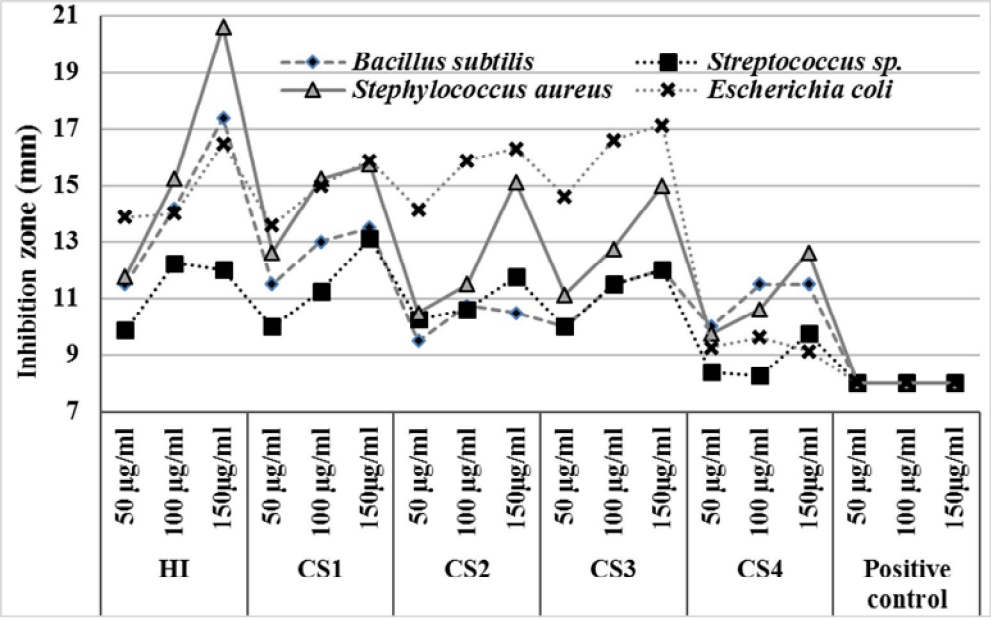

Fig. 1. Comparisons of antibacterial activities of the root extracts

Brine shrimp lethality bioassay is a useful test for toxicity screening of plant extracts because it is rapid and also inexpensive. A comparison was made for cytotoxic activity among the results of *H. indicus* and four commercial samples (Fig. 2). The percent (%) mortality of the brine shrimp nauplii increased as the concentration of the test solutions were increased. Apparently, the percent mortality of the nauplii was almost in the order of HI < CS4 < CS3 < CS2 < CS1 at different concentrations of the test solutions. This indicates that the mortality of the nauplii in sample extracts is concentration-dependent.

It was evident from the result that crude extract of HI had the highest toxicity against brine shrimp larvae as it showed LC_{50} values of 17.26 mg/ml, 47.54 mg/ml and 132.47 mg/ml after 6 hours, 12 hours and 24 hours respectively. Considering both LC_{50} and LC_{90} values, among the commercial samples, CS4 showed maximum cytotoxicity (33.37 µg/ml after 6hrs.) followed by CS3 (48.38 mg/ml after 6hrs). On the other hand, commercial sample 1 and 2 showed relatively poorer cytotoxicity to brine shrimp nauplii (Fig. 2).

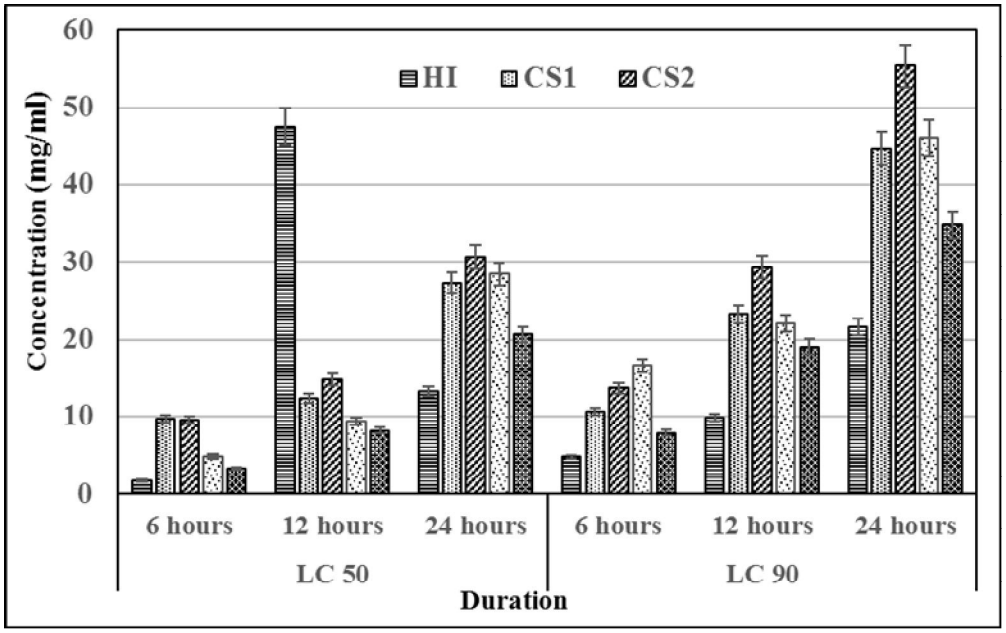

Fig. 2. Comparison of LC_{50} and LC_{90} value of five samples after 6, 12 and 24 hours

Krishnarajua *et al*. (2005) and Saha *et al.* (2013) studied the cytotoxic activity of different medicinal plants and found that the root extract of *H. indicus* was moderately toxic to brine shrimp nauplii. Saha *et al.* (2014) studied brine shrimp lethality bioassay of root extract of *H. indicus* and the LC_{50} value of methanolic extract was 2.55 µg/ml. In contrast, Lotufo *et al*. (2005) reported that *H. indicus* root extract did not show any cytotoxic activity to brine shrimp nauplii which supports the results of present study. It is postulated that extracts having LC_{50} values less than 1000 µg/ml are significantly non-toxic (Hossain *et al*., 2017). Thus, in the current study, all the test samples were found non-cytotoxic.

In the present study, the antioxidant potential was evaluated using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay at 1.25, 2.50, 5.00, 10.00, 50.00, and 100.00 µg/ml concentrations. The antioxidant activity of the authentic *H. indicus* root sample ranged between 4.78 to 88.36% followed by CS1, CS4 and CS1 with antioxidant activity values 5.98 to 83.44%, 4.6 to 80.13% and 4.89 to 79.97% respectively. Among all the samples, the highest antioxidant activity was observed as 88.36% in HI at 100 µg/ml concentration while the least activity was 4.1% observed in CS4 at 1.25 µg/ml concentration (Fig. 3). Notably, concentration dependent increase in antioxidant activity was observed in all the samples and scavenging activities of the commercial samples were in close proximity with HI.

Kumar *et al.* (2007) studied the antioxidant properties of terpenoid fraction of *H. indicus* and found the highest DPPH free radical scavenging activity as 84.72 % at 33.33 µg/ml

concentration which was close to the finding of the present study. Saha *et al*. (2014) studied the DPPH free radical scavenging activity of root extract of *H. indicus* and found that the scavenging activity of methanolic extract was 80.03% and 53.71% in 100 µg/ml and 50 µg/ml concentration respectively which corroborates with the findings of the current study.

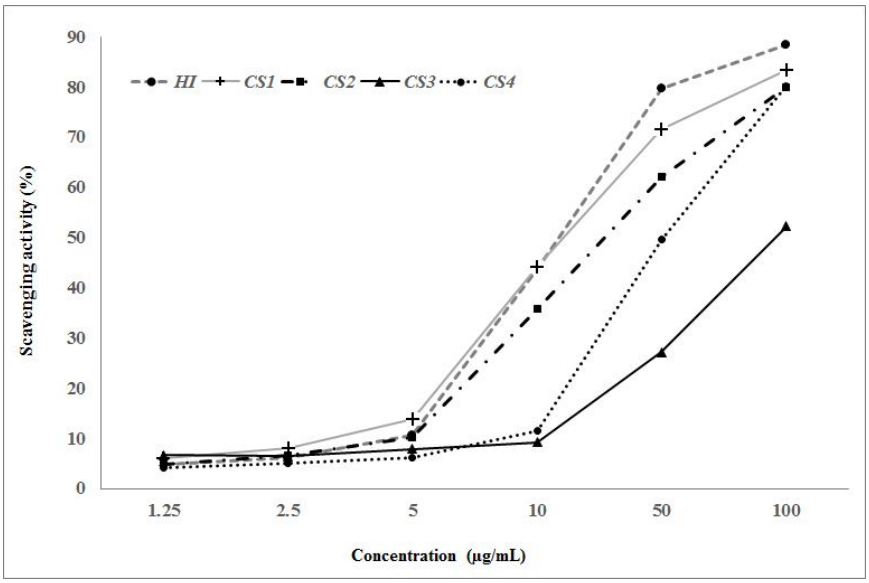

Fig. 3. Comparison of DPPH free radical scavenging activity of authentic and commercial root samples (CS) of *H. indicus* at different concentration

Antioxidant capacity can be associated directly to the phenolic content. The total phenolic content in authenticated anantamul root sample was 54.52 mg/ml gallic acid equivalent (GAE) per 100 mg plant extract. Moreover, the total phnolic contents in four commercial sample ranged between 52.28 to 75.37 mg/ml GAE per 100 mg extract (Fig. 4), which corroborates to the findings of Mandal *et al*. (2009). Mishra *et al*. (2018) reported on obtaining total polyphenolic content in *H. indicus* to be 45.5, 46.7, and 57.7 mg/g of extract in leaf, root, and stem, respectively and concluded that high concentration of phenolic compounds in the plant might be responsible for its strong antioxidant property. From the above mentioned results, it can be concluded that bioactivities of the commercial root samples varied with the root of known *H. indicus* which may be valuable to indicate the impurity in the anticipated root samples.

Fig. 4. Comparison of phenolic content of authentic and commercial root samples (CS) of *H. indicus* at different concentration

Herbal drug manufacturing companies in Bangladesh are widely using *H. indicus* for manufacturing many important Ayurvedic drugs such as Anantomul Salsa, Saribaddorist, Saribaddobusti, Arsagandarista and many more. Commercially available raw materials of *H. indicus* are collected from wild and therefore, there is a great chance of adulteration of this plant due to its high demand in the drug industries. Consequently, there is a greater chance of the real material being adulterated or substituted by similar looking cheaper material. Jeewandara *et al*. (2017) reported that there is a known tendency for deliberate adulteration of *H. indicus* by two other plants, *Cryptolepis buchananii* Roem. & Schult. (Periplocaceae) and *Ichnocarpus frutescens* (L.) R. Br. (Apocynaceae). Therefore, the findings of current research might be a decent implications in the field of phytochemistry and herbal medicine research.

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