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Screening of six Ayurvedic Medicinal Plant Extracts for Antioxidant and Cytotoxic Activity

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The antioxidant and cytotoxic activities of six medicinal plant crude extracts were evaluated in vitro by using different models. In DPPH scavenging method, highest scavenging of DPPH was displayed by *Hemidesmus indicus* with IC₅₀ values of 190.09 µg/ml while IC₅₀ values of the reference ascorbic acid were 18.80 µg/ml. However, in reducing power assay, the *Hemidesmus indicus* was found to demonstrate highest reducing power among these plants. Moreover, the *Hemidesmus indicus* was found to possess high amounts of phenolic content and the *Acalypha indica* was found to possess high amounts of flavonoid content, expressed as Gallic acid equivalents (GAE) and quercetin equivalents (QE) respectively. Importantly, the *Saussurea lappa* showed highest total antioxidant capacity and *Hemidesmus indicus* showed moderate total antioxidant capacity expressed as ascorbic acid equivalents (AAE). All the plant extracts were subjected to Brine Shrimp lethality bioassay where, *Saussurea lappa* was found highly toxic to brine shrimp nauplii, having LC₅₀ of 50.11 µg/ml while the LC₅₀ of the reference anticancer drug vincristine sulphate was 1.04 µg/ml.

Keyword: Ayurvedic medicine, Antioxidant, Cytotoxicity, Brine Shrimp Lethality Bioassay

1. Introduction

Traditional medicine system is based on different plants for many years around the world and still provides remedies in mankind^[1]. Active pharmaceutical compounds can be isolated from these plants which have good medicinal properties^[2]. According to World Health Organization (WHO, 1993), from 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlate directly with their traditional uses as plant medicine^[3]. In Bangladesh, most of the rural people rely on different forms of traditional medicine for their primary health care. Majority of the plants in Bangladesh did not undergo studies to investigate their bioactive

compounds^[4]. Therefore screening of Ayurvedic medicinal plant has potential in our country. Oxidative cell damaging caused by free radicals is the basis of many diseases^[5]. So intake of antioxidant is important for human. Plants are potential sources of natural antioxidants^[6]. In the present study we have been aimed at investigating the antioxidant properties of six plants by available and established antioxidants assay method like DPPH free radical scavenging method, reducing power assessment and total antioxidant capacity. Since phenolic and flavonoid photochemical exhibits strong antioxidant property, we also determined the phenolic and flavonoid content of the medicinal plant. We were also evaluated cytotoxic

properties by brine shrimp lethality bioassay as it seems to be a convenient, rapid and inexpensive method for the determination of the cytotoxic properties of the medicinal plant extracts^[7,8].

2. Description and medicinal uses

2.1 *Acalypha indica*

Acalypha indica is an erect, simple or branched, slightly hairy annual herb growing to a height of 40-80 cm. It is commonly known as Mukтажhuri, Swetbasanta (Bengali) and Indian acalypha (English). Results of leaf extract of *A. indica* showed promising larvicidal and ovicidal activity against malaria vector *A. stephensi*^[9]. Results of water extract study showed *A. indica* has neuroprotective and neurotherapeutic effects *ex vivo* on *m. gastrocnemius* frog^[10]. The ethanolic extracts of *Acalypha indica* were evaluated for their wound healing activity in rat^[11]. The acetone and ethanol extracts were found to be effective in causing significant antimicrobial activity^[12].

2.2 *Butea monosperma*

It is a medium sized dry season-deciduous tree, growing to 15 m tall commonly known as Palash, Dhak, Flame of the forest, Bastard Teak, Parrot Tree, Keshu (Punjabi) and Kesudo (Gujurati). The plant constituents have been reported to possess hepatoprotective^[13], anti-inflammatory^[14], antidiabetic^[15], antihelminthic^[16], antidiarrheal^[17], antifungal^[18] and antimicrobial^[19] activities.

2.3 *Capsicum annum*

Common names are red chilies (English), Lalmaricha (Hindi), Vattal mulaku, Kappal mulaku, Pachamulaku, Chuvanna mulaku (Malayalam). The plant constituents have been reported to possess antioxidant activity^[20], biological properties^[21] and antimicrobial properties^[22].

2.4 *Hemidesmus indicus*

Hemidesmus indicus is a twining shrub used as folk medicine and an ingredient in Ayurvedic and Unani preparations. It is known as Indian Sarsaparilla (English), Ananta, Gopasuta, Sariva (Sanskrit), Anantamul (Hindi), Ushbahindi

(Persian) and Irimusk (Sinhalese)^[23]. The root extract was used for preparing herbal soft drinks and food during famine^[24]. Various effects such as hypoglycemic^[25] and hypolipidemic^[26] have been reported. It mainly comprises saponins, tannins, hemidesmine, hemidesmol, hemidesterol, stearoptin, pregnane glycosides, β -sitosterol, indicusin, coumarin, volatile oils, triterpines, flavonoids, and so forth^[27].

2.5 *Saraca asoca*

Asoka, Asok, Vanjula, Sita asok, Hemapushpa, Gandhapushpa are some common names. It is used as anti-cancer medicinal source^[28]. *Saraca asoca* is reported to contain glycoside flavanoids, tannins, saponins, carbohydrate and proteins^[29].

2.6 *Saussurea lappa*

Common names are Costus and Kut root. The plant constituents have been reported to possess anti-inflammatory^[30] and cytotoxic activity^[31] of chloroform extract, antihepatotoxic^[32] activity of *Saussurea lappa* extract on D-galactosamine and lipopolysaccharide-induced hepatitis in mice. Phytochemical analysis^[33] of *Saussurea lappa* extract identified the presence of a biologically active compound guaianolide.

3. Materials and Methods

3.1 Collection and Identification

All plants were collected from different regions of Bangladesh. All of the samples were identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. The voucher specimen for all plant as *Capsicum annum* (DACB: 38136), *Saraca asoca* ((DACB: 38137), *Acalypha indica* ((DACB: 38138), *Hemidesmus indicus* (DACB: 38139), *Butea monosperma* (DACB: 38140) and *Saussurea lappa* (DACB: 38141) were deposited in the Herbarium.

3.2 Preparation of the plant samples

The collected and identified plant samples were dried in the sun and finally in a mechanical dryer at (60-70) °C. The dried samples were ground to coarse powder with a mechanical grinder and powdered samples were kept in clean closed glass containers pending extraction. During grinding of

sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other foreign matter deposited on the grinder.

3.3 Extraction of the Dried Powdered Samples

A glass made jar with plastic cover was taken and washed thoroughly. The jar was rinsed with ethanol and dried. Then the dried samples of all plants were taken in the jar. After that ethanol (800 ml) was poured into the jar up to 1 inch height above the sample surface as it can sufficiently cover the sample surface. The plastic cover with Aluminium foil was closed properly to resist the entrance of air into the jar. The process was performed for about 3 days. The jar was shaken in several times during the process to get better extraction.

3.4 Filtration and Concentration of the Extracts

After the extraction process the selected plant extracts were filtered with sterilized cotton filter. The cotton was rinsed with ethanol and fitted in a funnel. The filtrate was collected in a beaker. The plant extracts were concentrated by evaporating the solvent using a rotary evaporator.

3.5 Antioxidant activity test

3.5.1 DPPH free radical scavenging activity

DPPH was used to evaluate the free radical scavenging activity of plant extracts. 100µl of DPPH solution (0.4% w/v) was added to six test tubes made from stock solution of extract (100µg/ml) and was shaken by rotation. This mixture was kept at room temperature for 30 minutes in dark place. After 20 minutes of incubation, absorbance of the reaction mixture was taken at 517 nm using UV-visible spectrophotometer^[34].

3.5.2 Reducing Power Capacity Assessment

Reducing power capacity assessment was performed according to a standard method³⁵. 2.0 ml of plant extract (5µg/ml) was taken in a test tube. 2.5 ml of 1% Potassium Ferricyanide [$K_3Fe(CN)_6$] solution was added into the test tube. The test was incubated for 10 minutes at 50°C to

complete the reaction. 2.5 ml of 10% trichloro acetic acid solution was added into the test tube. The total mixture was centrifuged at 300 rpm for 30 minutes. 2.5 ml experimental solution was withdrawn from the mixture and mix with 2.5 ml of adjusted water and finally 0.5 ml of 0.1% ferric chloride solution were added. Then the absorbance of the solution was measured at 700 nm using a spectrophotometer against blank. The percentage (%) reducing capacity was calculated.

3.5.3 Determination of Total Antioxidant Capacity

The total antioxidant capacities of all plant extracts were evaluated following a described method^[36]. 300 µl of plant extract (5 µg/ml) or standard of different concentration solution was taken in a test tube. 3 ml of reagent solution (0.6M H_2SO_4 , 28mM sodium phosphate and 4mM ammonium molybdate) was added into the test tube. The test tube was incubated at 95°C for 90 minutes to complete the reaction. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. The antioxidant activity is expressed as the number of ascorbic acid.

3.5.4 Determination of Total Phenolic Content

About 1.0 ml of plant extract (5µg/ml) was taken in a test tube. Then 5 ml of Folin-ciocalteu (diluted 10 fold) reagent solution and 4 ml of sodium carbonate solution (7.5%) was added into the test tube. The test tube was incubated for 30 minutes at 20°C to complete the reaction. Then the absorbance of the solution was measured at 765 nm using spectrophotometer against blank. The total content of phenolic compounds in plant ethanol extracts in Gallic acid equivalents (GAE) was calculated.

3.5.5 Determination of Flavonoid Content

Total flavonoid contents of the plant extracts were determined according to the described method³⁷ using quercetin as a reference compound. 2 ml of plant extract (5µg/ml) in ethanol was taken in test tube. 200 µl of 10% Aluminium chloride solution and 1M potassium

acetate solution was added into the test tube. The test tube was incubated for 30 minutes at room temperature to complete the reaction. Then the absorbance of the solution was measured at 415 nm using a spectrophotometer against blank. The total content of flavonoid in plant extracts in quercetin equivalents was calculated.

3.6 Brine Shrimp Lethality Bioassay

3.6.1 Hatching of brine shrimp eggs

Artemia salina Leach (brine shrimp eggs) collected from the pet shop was used as the test organism. Simulated seawater was taken in the small tank and the shrimp eggs (1.5 g) were added to one side of the tank and this side was covered. The shrimps were allowed for two days to hatch and matured as nauplii (larvae). Constant oxygen supply was carried out during the hatching time. The hatched shrimps were attracted to the lamp light on the other side of the divided tank through the perforated dam. These nauplii were taken for this bioassay.

3.7 Preparation of the Test Sample Solution

Each sample (4.0 mg) was dissolved in 200 μ l of dimethyl sulfoxide (DMSO). A series of solutions of lower concentrations were prepared by serial dilution with DMSO. From each of these test solutions 100 μ l were added to the pre marked glass test tubes containing 5 ml of sea water (3.8% of sodium chloride) and 20 shrimp nauplii.

3.8 Preparation of Controls

Vincristine sulphate served as the positive control. 0.2 mg of vincristine sulphate was dissolved in DMSO to get an initial concentration of 20 μ g/ml from which serial dilutions were made using purely DMSO. The control groups containing 20 brine shrimp nauplii in 5 ml simulated sea water received the positive control solutions. As for negative control, 100 μ l of DMSO was added to each of three pre marked test tubes containing 5 ml of simulated sea water and 20 shrimp nauplii.

3.9 Application of Brine Shrimp Nauplii

With the help of the Pasteur pipette 20 living nauplii were added to each of the vials containing

5 ml of simulated sea water. A magnifying glass was used for convenient counting of the nauplii. For the control test of each vial, one vial containing the same volume of DMSO plus water up to 5 ml was used and 20 living nauplii were also taken in these control vials. Precautions were taken to avoid eggs during taking the shrimps in the vials.

3.10 Counting of Nauplii

After 24 h of incubation, the test tubes were observed using a magnifying glass and the number of survived nauplii in each vial were counted and recorded. From this data, the percentage of mortality of the nauplii was calculated for each concentration of the sample.

4. Results and Discussion

4.1 DPPH Free Radical Scavenging Activity

The results of DPPH free radical scavenging activity of the plant extracts is shown in the figure 1, where % scavenging of DPPH radical was found to rise with increasing concentration of the different plants with highest scavenging displayed by *Hemidesmus indicus*.

4.2 Reducing Power

Reducing power of different plant extracts were assessed using ferric to ferrous reduction activity as determined spectrophotometrically from the formation of Perl's Prussian blue colored complex^[38]. Reducing power was found to increase with increasing concentration of the extracts in all cases (Fig 2).

4.3 Total Antioxidant Capacity

Total antioxidant capacity of the plant was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per of plant extract (Table 1). Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid ($y = 0.006x + 0.098$; $R^2 = 0.990$). Values are the mean of duplicate experiments and represented as mean \pm SD.

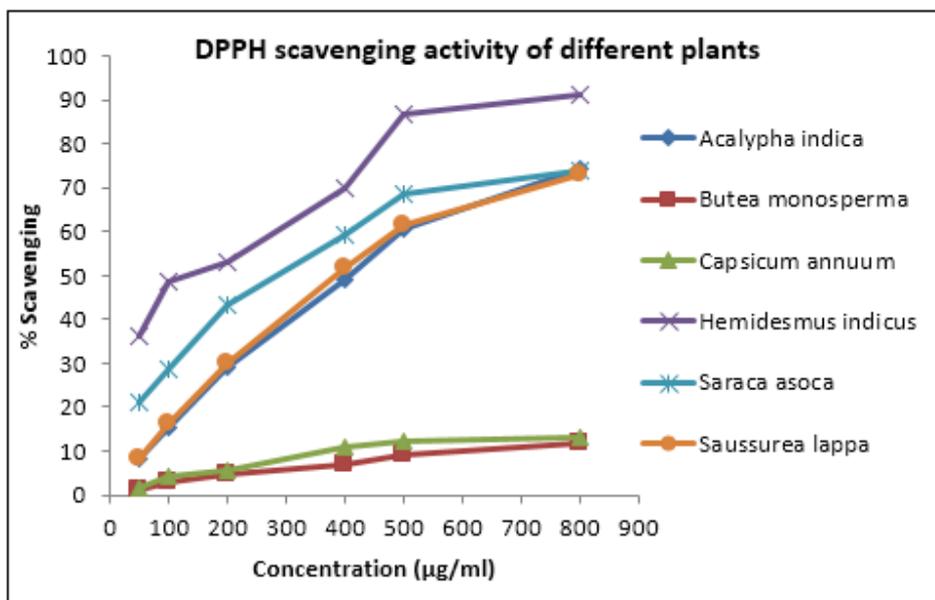


Fig 1: DPPH scavenging activities of different plant extracts

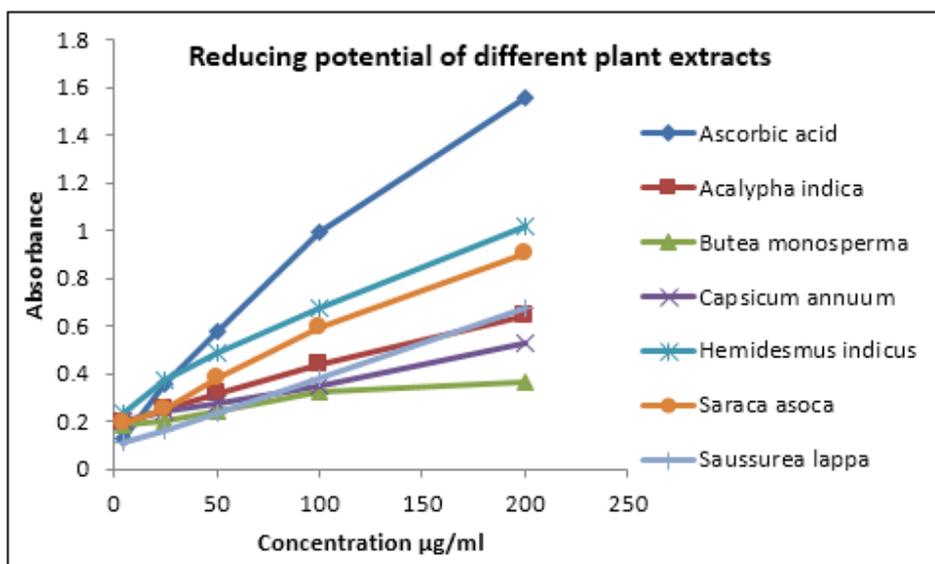


Fig 2: Reducing power of different plant extracts

4.4 Total Phenol Content Determination

Total phenolic contents of the plant extracts were determined by using the Folin-Ciocalteu reagent and were expressed as Gallic acid equivalents (GAE) per gram of plant extracts (Table 1). The total phenolic contents of the test of above plants were calculated using the standard curve of Gallic acid ($y = 0.011x + 0.122$; $R^2 = 0.990$).

4.5 Determination of Total Flavonoid Content

Aluminium chloride colorimetric method was used to determine the total flavonoid contents (Table 1). The total flavonoid content was calculated using the standard curve of quercetin ($y = 0.005x + 0.005$; $R^2 = 0.996$) and was expressed as quercetin equivalents (QE) per gram of the plant extract.

4.6 Brine Shrimp Lethality Bioassay

All the plant extract were subjected to Brine Shrimp lethality bioassay for possible cytotoxic action. In this study, *Saussurea lappa* was found to be showed satisfactory toxic to Brine Shrimp nauplii, with LC_{50} value of 50.11 $\mu\text{g/ml}$ whereas anticancer drug vincristine sulphate's LC_{50} value was 1.04 $\mu\text{g/ml}$. However, *Acalypha indica*, *Saraca asoca* and *Hemidesmus indicus* were found to be moderately cytotoxic showing LC_{50}

60.26 $\mu\text{g/ml}$, 117.49 $\mu\text{g/ml}$ and 177.83 $\mu\text{g/ml}$ respectively. On the other hand, *Butea monosperma* and *Capsicum annum* were found to be showing LC_{50} 208.92 $\mu\text{g/ml}$ and 346.73 $\mu\text{g/ml}$ respectively. All plants produced concentration dependent increment in percent mortality of Brine Shrimp nauplii indicating the presence of cytotoxic principles in these extractives (Fig 3).

Table 1: Total anti oxidant, phenol and flavonoid content of plant extracts

Samples	Total antioxidant capacity (mg/g ascorbic acid equivalent)	Total phenol content (mg/g Gallic acid equivalent)	Total flavonoid content (mg/g quercetin equivalent)
<i>Acalypha indica</i>	92.5 \pm 5.89	13.5 \pm 2.57	49.5 \pm 0.71
<i>Butea monosperma</i>	10.42 \pm 0.59	7.05 \pm 0.96	5.0 \pm 0
<i>Capsicum annum</i>	56.25 \pm 17.09	31.36 \pm 12.21	13.5 \pm 0.71
<i>Hemidesmus indicus</i>	137.916 \pm 1.77	118.41 \pm 12.53	5.0 \pm 0
<i>Saraca asoca</i>	236.67 \pm 51.85	73.87 \pm 8.03	10.0 \pm 0
<i>Saussurea lappa</i>	340.0 \pm 4.71	31.59 \pm 4.18	5.0 \pm 0

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

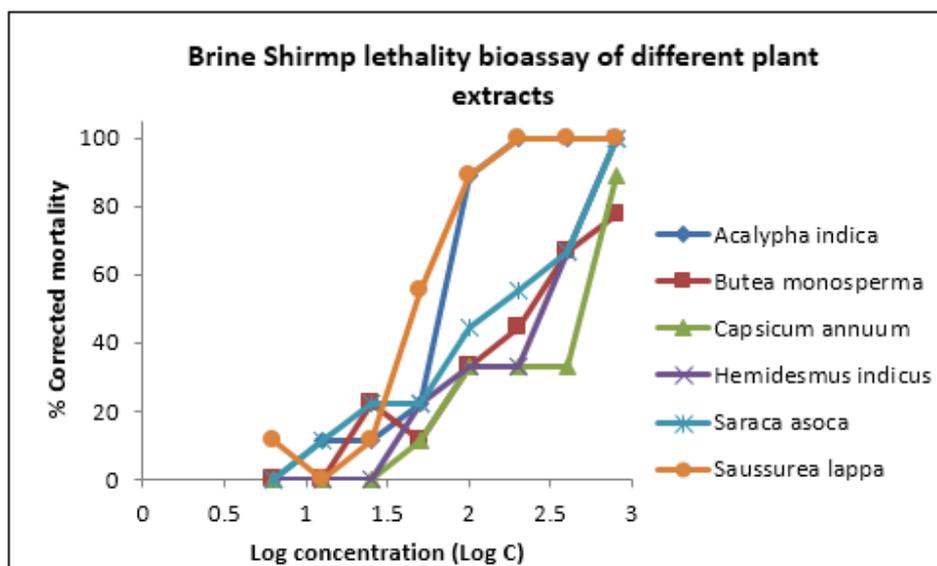


Fig 3: Cytotoxic potential of different plant extracts

5. Conclusion

The ethanolic extracts of *Acalypha indica*, *Butea monosperma*, *Capsicum annum*, *Hemidesmus indicus*, *Saraca asoca* and *Saussurea lappa* plants traditionally used in Ayurvedic medicine were screened for antioxidant and cytotoxic activity in this study. The antioxidant activities of these plant crude extracts were evaluated using different models in vitro, including DPPH radical

scavenging assay, total antioxidant capacity, total phenol content determination, total flavonoid content determination, reducing power and cytotoxic activities were evaluated by brine shrimp lethality bioassay. The rank of cytotoxic potential of the test samples were as follows Vincristine sulphate > *Saussurea lappa* > *Acalypha indica* > *Saraca asoca* > *Hemidesmus*

indicum > *Butea monosperma* > *Capsicum annumum*.

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