Research Article

Cytotoxicity Potentials of Eleven Bangladeshi Medicinal Plants

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Various forms of cancer are rising all over the world, requiring newer therapy. The quest of anticancer drugs both from natural and synthetic sources is the demand of time. In this study, fourteen extracts of different parts of eleven Bangladeshi medicinal plants which have been traditionally used for the treatment of different types of carcinoma, tumor, leprosy, and diseases associated with cancer were evaluated for their cytotoxicity for the first time. Extraction was conceded using methanol. Phytochemical groups like reducing sugars, tannins, saponins, steroids, gums, flavonoids, and alkaloids were tested using standard chromogenic reagents. Plants were evaluated for cytotoxicity by brine shrimp lethality bioassay using \textit{Artemia salina} comparing with standard anticancer drug vincristine sulphate. All the extracts showed potent to moderate cytotoxicity ranging from \( \text{LC}_{50} \) 2 to 115 \( \mu \text{g/mL} \). The highest toxicity was shown by \textit{Hygrophila spinosa} seeds (\( \text{LC}_{50} = 2.93 \mu \text{g/mL} \)) and the lowest by \textit{Litsea glutinosa} leaves (\( \text{LC}_{50} = 114.71 \mu \text{g/mL} \)) in comparison with standard vincristine sulphate (\( \text{LC}_{50} = 2.04 \mu \text{g/mL} \)). Among the plants, the plants traditionally used in different cancer and microbial treatments showed highest cytotoxicity. The results support their ethnomedicinal uses and require advanced investigation to elucidate responsible compounds as well as their mode of action.

1. Introduction

Nature is the source of 87% of drugs used to treat all categorized human diseases. 25% of prescribed drugs originated from plants. Over 3000 species of plants have been reported for their anticancer property. Uddin et al. [1] stated that till now about 80% people in developing countries rely on traditional plant based medicines for their primary health care. Focus on natural products is increasing day by day as it serves as an enormous source of new anticancer drugs. According to Washart [2] natural products are vital in the treatment of cancer, as a number of important anticancer agents have been derived from natural products, including plant-derived agents such as the vinca alkaloids, taxanes, and topoisomerase I inhibitors. According to intercontinental marketing services report [3], the anticancer drug market in Bangladesh is growing at 20 percent a year due to an alarming rise in cancer patients. So, search for new anticancer drugs is the demand of time.

Bangladesh has a rich and prestigious heritage of herbal medicines among the South Asian countries. Ghani [4] estimated that about 250 species of medicinal plants are used for the preparation of traditional medicines which is the half of total species of plants grown in Bangladesh. However, the majority of these plants have not yet undergone chemical, pharmacological, and toxicological studies to investigate their bioactive compound(s). Traditional resources and ecological diversity indicate that Bangladeshi plants represent an exciting resource for new drug discovery.

Ahmed and Uddin [5, 6] identified \textit{Melastoma malabathricum} L., synonym \textit{M. polyanthum}, as a spreading shrub; leaves are opposite and lanceolate; flowers are solitary...
clustered, purple in color. The plant grows in the hill tracts and also in roadside of central and east region of Bangladesh. It is also found in Thailand, India, and Sri Lanka. Many researchers [7–10] recognized this plant as an ethnomedical plant in various countries of the world. M. malabaricum is reported as antiviral by Nazлина et al. [11], antibacterial by Choudhury et al. [12], antioxidant and cytotoxic by Alwash et al. [13], antinociceptive, anti-inflammatory, and antipyretic by Zakaria et al. [14], antidiarrhoeal by Sunilson et al. [15], antidiabetic and antihyperlipidemic by Kumar et al. [16], and hepatoprotective by Mamata et al. [17]. Sirat et al. [18] isolated amides, triterpenes, and flavonoids.

Strychnos nux-vomica (L.) Roxb., synonym L. chinensis, is medium-sized deciduous or semi-evergreen tree; leaves are aromatic, ovate, or elliptic. The plant grows in Bangladesh, India, Sri Lanka, Myanmar, China, and Malaysia as described by Uddin [6]. Mandal, Yangon, China, and Malaysia as described by Uddin [6]. Mandal et al. [19] reported antimicrobial, Kar et al. [20] cardiovascular, and Menon et al. [21] psychopharmacological activity. Agrawal et al. [22] isolated four butenolides, Wang et al. [23] 2′-oxygenated flavone glycoside, and Herath et al. [24] D-xylene and L-arabinose from the plant.

Malpighia coccigera is not locally considered as a medicinal plant; rather it is an ornamental hedge plant. Seipold et al. [25] isolated floral oil from this plant. Evans [26] considered that other species of Malpighia are hallucinogenic.

Baker [27] identified Pseudelephantopus spicatus L. as a herb; hairs are present on both the upper and lower surfaces of the leaf blade, flowers are white borne in narrow bullet-shaped heads, four flowers per head, and the pappus is present on the end of each fruit. Odonne et al. [28] isolated three compounds: two hirsutinolides and ursolic acid having activity against Leishmania amazonensis from P. spicatus.

According to Uddin [29], Viscum orientale Willd., synonym V. verticillatum Roxb., is semiparasitic much-branched shrub on trees; leaves are opposite, thick, elliptic, or obovate, rarely lanceolate, obtuse, and glabrous, flowers are in axillary sessile or slightly pedunculate, acute, deciduous, and berry is ovoid or subglobose. The plant grows on various species of trees at low and medium altitudes forests of Chittagong, Chittagong hill tracts, and Sundarban mangrove forest in Bangladesh. It also occurs in India (Bihar, West Bengal, and Kerala). Schneeweis [30] stated that the plant parasitizing Strychnos nux-vomica tree is used in Indian medicine. Satish et al. [31] reported the parasite for antibiotic activity. Lee et al. [32] evaluated another species, V. album for inhibitory activities of pancreatice lipase and phosphodiesterase. Park et al. [33] reported that V. album contains beta-galactoside and N-acetyl-D-galactosamine-specific lectin II (60 kDa), polysaccharides, and viscotoxin (5 kDa) with their antitumor activity.

Naser et al. [34] described that Thuja occidentalis L. is indigenous to eastern North America and is grown in Europe as an ornamental tree with a maximal height. It has coniferous pyramidal features, with flattened branches and twigs in one plane, bearing small scale-like leaves. Over the whole year, the leaves are green, with the lower side showing a brighter green where resin glands also reside. Small coniferous pins contain the seeds. Kumar et al. [35] reported antibacterial, anticancer, anti-HIV, antispasmodic, antioxidant, antidiabetic, hepatoprotective, insecticidal, radioprotective, antiatherosclerosis, and neuropharmacological and Naser et al. [34] anti-influenza activities of this plant.

Ahmed [5] described Hoya parasitica (Roxb.), synonym Asclepias parasitica, as a tall climber; its stems are stout or slender, glabrous. Its leaves are ovate elliptic or lanceolate acute or acuminate peduncles are solitary or in pairs short or long slender or stout, pedicels slender long glabrous, coronal-processes longer than the corolla tube; the plant bears aesthetic flowers in May to June. It is mostly grown parasite on giant trees, found in the Chittagong, Sylhet, and Satkhira districts and in the Sundarbans of Bangladesh. It also grows in Assam, East Bengal, Tapera, Crounilla, Malacca, Singapore, and the Andaman Island. One of the same authors of this study [36,37] reported the antibacterial and antinociceptive activities of Hoya parasitica leaves and growth inhibitory effects of dihydrocanaric acid against both HeLa and SW480 cells. Mukherjee et al. [38] reported the plant to contain triterpenic 3,4-seco acid 3,4-secolup-20(29)-en-3-oic acid, along with lupeol and lupenone from stem and Sadhu et al. [37] reported to contain an androstanoid, a sesquiterpene, and a phenolic compound, together with a known triterpene, dihydrocanaric acid.

Ahmed [5] stated that Cnicus arvensis (L.) Roth., synonym Cirsi num arven se (L.). Scop., is an erect herb; leaves are alternate; flower heads are solitary, hermaphrodite, pappus, and purple. It grows in sandy soils, river banks, and rice fields in Bangladesh. No scientific study has been reported yet.

According to Ghani, Ahmed, and Hooker Sir [4,5,39], Commelina benghalensis Linn. is a pubescent and ascending herb which grows all over Bangladesh. The plant is reported for its antimicrobial by Khan et al. [40], sedative and anxiolytic by Hasan et al. [41], and anticancer activity by Mbazima et al. [42].

Baccaurea ramiflora Lour., synonym B. sapida (Roxb.) Muell.-Arg., is described by Brandis [43]. It is a semievergreen tree; fruit is yellowish and velvety with pinkish white pulp. M. Sundriyal and R. C. Sundriyal [44] stated that it is native to Southeast Asia region. The plant has antioxidant property which was reported by Goyal et al. [45]. Yang et al. [46] isolated vanillloid derivatives from this plant.

Ahmed [5] described that Hygrophila spinosa T. Ander, synonym H. auriculata, is a stout, erect herb covered with stiff hair, leaves are opposite and lanceolate; flowers are in axillary whorls and purple. It grows throughout the plain districts of Indian subcontinent, in dump areas such as marshy margins of canals, rice fields, and so forth. It is also seen in tropical Himalaya, Ceylon, Myanmar, Indochina, and Malaya. Gomes et al. [47] reported the plant for its haematinic, Mazumdar et al. [48] for antitumor, and Kumari and Iyer [49] for diuretic activity. Previously any class of constituents was not reported. Folklore uses of these plants are summarized in Table 1.

2. Materials and Methods

2.1. Collection and Identification of Plant Material. The different parts of eleven plants were collected from different parts of Bangladesh. The samples of the plants were mounted
Table 1: Folklore uses of the studied eleven plants.

| Species                        | Family            | Traditional use                                                                                                                                                                                                                                                                                                                                 |
|--------------------------------|-------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Baccaurea ramiflora Lour.      | Euphorbiaceae     | Uddin [6] stated that the plant is used in diarrhoea, flatulence, gastric ulcer, ureterolithiasis, and jaundice.                                                                                                                                                                                                                               |
| Cnicus arvensis (L.) Roth.     | Asteraceae        | Ahmed [5] indicated that the plant is used as cholagogic and diuretic. Uddin [6] wrote that traditionally it is used in the treatment of cirrhosis, diabetes, excessive menstruation, gout, hyperacidity (gastritis), liver cancer, jaundice, and scabies.                                     |
| Commelina benghalensis Linn.   | Commelinaceae     | According to Ahmed [5], the plant has reputation to be used as antiseptic, demulcent, emollient, and refrigerant. Ghani [4] stated that it is also used in leprosy.                                                                                                                                        |
| Hoya parasitica (Roxb.)        | Asclepiadaceae    | Ahmed [5] credited leaves of this plant to treat rheumatism.                                                                                                                                                                                                                                                                                   |
| Hygrophila spinosa T. Ander    | Acanthaceae       | Ahmed [5] described that the leaves, seeds, and roots are traditionally used as diuretic and also for jaundice, rheumatism, diseases of urogenital tract, and bladder stones. Joshi [50] and Kapoor and Mitra [51] reported that it is a reputed remedy for arthritis. It is also used as aphrodisiac, roborant, demulcent, and diuretic. The plant is useful in cancer and tubercular fistula and juice in anaemia. It is the source of locally used Ayurvedic, Unani, and indigenous drug preparations having anabolic-cum androgen-like activity. |
| Litsea glutinosa (Lour.) Roxb. | Lauraceae         | Uddin [6] reported that the plant is used in the treatment of ankilitis, asthma, bone fracture, tumor, leucorrhoea, hook worm infestation, rheumatoid arthritis, jaundice, epilepsy, liver disease, and dysentery.                                                                                                           |
| Malpighia coccigera            | Malpighiaceae     | Not found.                                                                                                                                                                                                                                                                                                                                   |
| Melastoma malabathricum L.     | Melastomataceae   | Kabir et al. [52] reported that this plant is used among the Tripura tribes in Bangladesh for the treatment of jaundice. Ahmed [5] pointed that the plant is used in diarrhoea, dysentery, wound healing, and skin diseases and Uddin [6] credited the plant for use in abdominal pain, sores in tongue, oedema, gynecological diseases, and snake bite. |
| Pseudelephantopus spicatus L.  | Asteraceae        | Odone et al. [28] reported that the plant is used by the ethnic group from the Peruvian Amazonia in leishmaniasis.                                                                                                                                                                                                                               |
| Thuja occidentalis L.           | Cupressaceae      | According to Naser et al. [34] and Kumar et al. [35], in folk medicine, T. occidentalis has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhoea, external fungal infections of the skin (ringworm and thrush), headache, scurvy prevention, eczema, anal or genital warts, and rheumatism. It is used as abortifacient, emmenagogue, vermifuge, diuretic, and digestive aid. |
| Viscum orientale Willd.         | Loranthaceae      | Stuar [53] the plant was considered poisonous in folklore medicine; in India used as a substitute for nux-vomica and used for postular itches. Leaves are burned to ashes which are then mixed with sulphur and coconut oil and rubbed on the body. Poultice is used in neuralgia in Bangladesh. Nayak et al. [54] stated that the plant is used in giddiness and stiffness in Orissa, India. |
Table 2: Botanical information of eleven medicinal plants of Bangladesh.

| Species                     | Family           | Local name | English name     | Collection area | Collection time | Acc. number |
|-----------------------------|------------------|------------|------------------|-----------------|-----------------|-------------|
| Baccaurea ramiflora         | Euphorbiaceae    | Latkan     | Burmese grape    | Narsingdi       | August          | DACB-35863  |
| Cnicus arvensis             | Asteraceae       | Birhalkanta| Creeping Thistle | Khulna          | March           | DACB-31,544  |
| Commelina benghalensis      | Commelinaceae    | Kanchire   | Benghal dayflower| Dhaka           | November        | DACB-32,495  |
| Hoya parasitica             | Asclepiadaceae   | Bayupriya  | Wax flower       | Satkhira        | April           | DACB-30,224  |
| Hygrophila spinosa          | Acanthaceae      | Talmakhana | Marsh barbel     | Satkhira        | July            | DACB-31257  |
| Litsea glutinosa (Lour.) Roxb. | Lauraceae     | Kukur chita| Bollygum         | Munshiganj      | May             | DACB-25734  |
| Malpighia coccigera         | Malpighiaceae    | Khoiphool  | Dwarf Holly      | Dhaka           | January         | DACB-37527  |
| Melastoma malabathricum L. | Melastomataceae  | Dantrasha  | Malabar melastome| Savar, Dhaka    | July            | DACB-15161  |
| Pseudelephantopus spicatus L.| Asteraceae  | Kukur jihba| Dog’s tongue     | Satkhira        | January         | DACB-35,012  |
| Thuja occidentalis          | Cupressaceae     | Thuja      | White cedar      | Dhaka           | June            | DACB-37930  |
| Viscum orientale Willd.     | Loranthaceae     | Banda      | Mistletoe        | Satkhira mangrove| July          | DACB-38174  |

a Accession number.

Table 3: Phytochemical evaluation of the fourteen extracts of eleven medicinal plants of Bangladesh.

| Plants                     | Part used   | % yield | Red. sug. | Alk. | Str. | Tan. | Gum | Flv. | Sap. |
|----------------------------|-------------|---------|-----------|------|------|------|-----|------|------|
| Baccaurea ramiflora        | Leaves      | 5.00    | –         | +    | +    | +    | +   | –    | –    |
| Baccaurea ramiflora        | Bark        | 3.00    | +         | –    | +    | –    | –   | –    | –    |
| Cnicus arvensis            | Aerial part | 3.17    | ND        | ND   | ND   | ND   | ND  | ND   | ND   |
| Commelina benghalensis     | Aerial part | 3.00    | ND        | ND   | ND   | ND   | ND  | ND   | ND   |
| Hoya parasitica            | Stem        | 3.08    | +         | +    | –    | –    | +   | –    | –    |
| Hygrophila spinosa         | Seeds       | 12.0    | +         | +    | –    | –    | +   | +    | +    |
| Litsea glutinosa (Lour.) Roxb. | Leaves    | 2.90    | –         | +    | +    | –    | –   | –    | –    |
| Malpighia coccigera        | Leaves      | 4.92    | +         | –    | +    | –    | –   | –    | +    |
| Melastoma malabathricum L.| Leaves      | 9.09    | +         | +    | +    | –    | –   | –    | –    |
| Melastoma malabathricum L.| Stem        | 3.22    | +         | –    | +    | –    | –   | –    | –    |
| Pseudelephantopus spicatus L.| Aerial part| 3.4     | –         | +    | +    | –    | –   | +    | –    |
| Thuja occidentalis         | Leaves      | 2.57    | –         | +    | +    | –    | –   | –    | –    |
| Thuja occidentalis         | Bark        | 5.25    | +         | +    | +    | –    | –   | –    | –    |
| Viscum orientale Willd.    | Aerial part | 4.35    | +         | –    | +    | –    | –   | –    | –    |

a Reducing sugar, b alkaloid, c steroid, d tannin, e flavonoid, f saponin, and g not done.

on herbarium sheet and the species were taxonomically confirmed by Sarder Nasir Uddin, Principle Scientific Officer, Bangladesh National Herbarium (BNH), Mirpur, Dhaka. The voucher specimens of the plants have been deposited and preserved in BNH library for further collection and reference (Table 2).

2.2. Preparation of Methanol Extract. The collected different plant parts were separated from undesirable materials. They were dried in open air for two weeks. The shade dried plant parts were ground into a coarse powder with the help of a suitable grinder (capacitor start motor, Wuhu motor factory, China). The powders of the plant parts were stored in an air-tight container and kept in a cool, dark, and dry place until the analysis commenced. Powered materials were taken in some clean, flat-bottomed glass containers and soaked in methanol. The containers along with their contents were sealed and kept for a period of 10 days with occasional shaking or stirring. The mixtures then underwent a coarse filtration by cotton and Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrates were concentrated under air. Different amounts of concentrate extracts were obtained which were designated as crude methanol extracts (Table 3). Extraction was conceded following the method depicted by Khatun et al. [55].

2.3. Chemicals and Reagents. Standard chromogenic reagents lead acetate, potassium dichromate, ferric chloride, hydrochloric acid, sulfuric acid, Mayer’s reagent, Dragendorff’s reagent, Wagner’s reagent, Hager’s reagent, Molisch reagent, Benedict’s reagent, and Fehling’s solutions used for preliminary phytochemical chemical group test were of reagent grade and purchased from Sigma-Aldrich Co. LLC, Missouri, United States. Vincreistine sulfate, used as a standard drug in the cytotoxic assay, was collected from the Techno Drugs Limited, Bangladesh. Methanol supplied by Laboratory Patterson Scientific, UK, was used as solvent for maceration of the plant material. Dimethyl sulfoxide (DMSO, ≥ 99.9%, BioReagent, for molecular biology; Sigma-Aldrich, India) was used to dissolve the extracts.

2.4. Instruments and Equipment. Electronic balance (serial number 1508, OHAUS, Germany) was used for this study.
Glass-made hatching tank, air pump, and cover lamp to grow shrimp were purchased locally. Pipettes, micropipette, test tubes, and other glass apparatus used were of laboratory standard and procured from authorized dealer.

2.5. Test for Different Chemical Groups. The preliminary standard and procured from authorized dealer. tubes, and other glass apparatus used were of laboratory shrimp were purchased locally. Pipettes, micropipette, test glass-made hatching tank, air pump, and cover lamp to grow

a Median lethal concentration.

Fourteen extracts of eleven medicinal plants were evaluated for their cytotoxicity. Among them four extracts, aerial part of Cnicus arvensis, Pseudelephantopus spicatus, and Viscum orientale leaves, and Hygrophila spinosa seeds showed potent cytotoxicity LC\textsubscript{50} ranging from 2 to 22 \(\mug/mL\) in comparison with standard vincristine sulfate (LC\textsubscript{50} = 2.04 \(\mug/mL\)). Other plants also showed quite high cytotoxicity LC\textsubscript{50} ranging from 21 to 115 \(\mug/mL\) (Table 4). The highest cytotoxicity was found in H. spinosa seeds (LC\textsubscript{50} = 2.93 \(\mug/mL\)) and the lowest in Litsea glutinosa leaves (LC\textsubscript{50} = 114.71 \(\mug/mL\)). We evaluated two plants of the family Asteraceae (C. arvensis and P. spicatus) and both of the plants showed significant cytotoxicity. Leaves of T. occidentalis showed significant cytotoxicity where the bark of the plant showed moderate cytotoxicity. T. occidentalis, H. spinosa, and C. arvensis are used ethnomedicinally in the treatment of cancer. In our investigation, we found the highest cytotoxicity of these plants. But L. glutinosa leaves showed the lowest cytotoxicity though it is traditionally used in treatment of tumor. On the other hand, Malpighia coccigera is not recognized as traditional medicinal plant but showed significant cytotoxicity (LC\textsubscript{50} = 35.96 \(\mug/mL\)). L. glutinosa and M. coccigera may be tested for their cytotoxicity by other method to evaluate the present result. Melastoma malabathricum, L. glutinosa, P. spicatus, Viscum orientale, and Commelina benghalensis are traditionally reputed as either antimicrobial agent or poisonous plant. Cytotoxicity of Melastoma malabathricum was evaluated earlier in a process other than this by Alwash et al. [13] and anticancer activity of Hoya parasitica is previously reported by Sadhu et al. [37].
4. Conclusion

According to Sagar et al. [58], a master herbalist can advise on potential herbal treatments derived from centuries of traditional observations and advanced traditional medical systems such as Ayurveda. It will be imperative to develop a new model of modern pharmacology based on traditional pharmacognosy. Traditionally used plants in cancer treatments proved their efficacy in different pathway like inhibition of angiogenesis and metastasis, induction of apoptosis, and so forth. The plants showing significant cytotoxicity can be investigated for their bioactive compounds and their mode of action. Compounds may be isolated from plants showing significant cytotoxicity to identify the cytotoxic compounds and elucidate the possible mode of action using suitable technique. Most of the cytotoxic drugs possess serious adverse effect and their efficacy is unpredictable. New cytotoxic compounds found from these plants may present us a group of new well-tolerated anticancer and antimicrobial drugs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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