Original Article

Cytotoxic Effects of Bangladeshi Medicinal Plant Extracts

Shaikh J. Uddin,1 I. Darren Grice,2 and Evelin Tiralongo1

1 School of Pharmacy, Griffith University, Gold Coast campus, 4222, Queensland, Australia
2 Institute for Glycomics, Griffith University, Gold Coast campus, Australia

Correspondence should be addressed to Evelin Tiralongo, e.tiralongo@griffith.edu.au

Received 1 December 2008; Accepted 21 July 2009

Copyright © 2011 Shaikh J. Uddin et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

To investigate the cytotoxic effect of some Bangladeshi medicinal plant extracts, 16 Bangladeshi medicinal plants were successively extracted with n-hexane, dichloromethane, methanol and water. The methanolic and aqueous extracts were screened for cytotoxic activity against healthy mouse fibroblasts (NIH3T3) and three human cancer-cell lines (gastric: AGS; colon: HT-29; and breast: MDA-MB-453) using the MTT assay. Two methanolic extracts (Hygrophila auriculata and Hibiscus tiliaceous) and one aqueous extract (Limnophila indica) showed no toxicity against healthy mouse fibroblasts, but selective cytotoxicity against breast cancer cells (IC50 1.1–1.6 mg mL−1). Seven methanolic extracts from L. indica, Clerodendron inerme, Cynometra ramiflora, Xylocarpus moluccensis, Argemone mexicana, Ammannia baccifera and Acrostichum aureum and four aqueous extracts from Hygrophila auriculata, Bruguiera gymnorrhiza, X. moluccensis and Aegiceras corniculatum showed low toxicity (IC50 > 2.5 mg mL−1) against mouse fibroblasts but selective cytotoxicity (IC50 0.2–2.3 mg mL−1) against different cancer cell lines. The methanolic extract of Blumea lacera showed the highest cytotoxicity (IC50 0.01–0.08 mg mL−1) against all tested cell lines among all extracts tested in this study. For some of the plants their traditional use as anticancer treatments correlates with the cytotoxic results, whereas for others so far unknown cytotoxic activities were identified.

1. Introduction

Natural products and related drugs are used to treat 87% of all categorized human diseases including bacterial infection, cancer and immunological disorders [1]. About 25% of prescribed drugs in the world originate from plants [2] and over 3000 species of plants have been reported to have anticancer properties [3]. About 80% of the population in developing countries rely on traditional plant based medicines for their primary health care needs [4]. Bangladesh has a rich and prestigious heritage of herbal medicines among the South Asian countries. More than 500 species of medicinal plants are estimated as growing in Bangladesh and about 250 species of them are used for the preparation of traditional medicines. However, the majority of these plants have not yet undergone chemical, pharmacological and toxicological studies to investigate their bioactive compound(s) [5]. Traditional records and ecological diversity indicate that Bangladeshi plants represent an exciting resource for possible lead structures in drug design.

In this study, 16 plants (Adiantum caudatum, Ammannia baccifera, Argemone mexicana, Blumea lacera, Clerodendron inerme, Ficus religiosa, Hygrophila auriculata, Limnophila indica and Mollugo pentaphylla) including seven mangrove species (Acrostichum aureum, Aegiceras corniculatum, Bruguiera gymnorrhiza, Cynometra ramiflora, Hibiscus tiliaceous, Pandanus foetidus and Xylocarpus moluccensis) were collected from tidal forests in the coastal Sundarbans (a swamp region in the Ganges delta) and other locations in the Khulna district of Bangladesh to screen them for possible cytotoxic activity. Except Cynometra ramiflora, all of these plants have been used in traditional medicine of Bangladesh for the treatment of various diseases such as cancer, inflammation or infectious diseases (Table 1) [5, 6]. Only limited research has been performed on these plants to evaluate their anticancer potential. In previous studies using extracts from Hygrophila auriculata, Bruguiera gymnorrhiza, Clerodendron inerme, Blumea lacera, Hibiscus tiliaceous and Argemone mexicana NFκ-B inhibition, cytotoxic or cytoprotective activities have been observed [7–15]. For other plants (i.e., Clerodendron inerme, M. pentaphylla and Aegiceras corniculatum) anti-inflammatory activity [16–18], anti-oxidant activity (Hygrophila auriculata, Bruguiera gymnorrhiza, X. moluccensis and Hibiscus tiliaceous) [19–22]
Table 1: List of selected Bangladeshi medicinal plants with their traditional uses.

| Plant species              | Family          | Local name  | Voucher     | Traditional uses                                      |
|---------------------------|-----------------|-------------|-------------|------------------------------------------------------|
| *Acrostichum aureum*     | Pteridaceae     | Tiger fern  | DACB 31538  | R: rheumatism, treat wounds and boils; L: used to stop bleeding |
| *Adiantum caudatum*      | Adiantaceae     | Mayurshikha | DACB 31268  | L: expectorant, antipyretic, diabetes, skin disease; WP: antibacterial, hypoglycaemic |
| *Aegiceras corniculatum* | Myrsinaceae     | Kholisha    | DACB 31584  | B: fish poison, asthma, diabetes and rheumatism       |
| *Ammannia bacifera*      | Lythraceae      | Jangli mendi | NA         | L: rheumatism, skin diseases, ring worm and fever      |
| *Argemone mexicana*      | Papaveraceae    | Shialkata   | DACB 30213  | L: antifungal, antiviral, antihelminthic, syphilitic infection, dysentery |
| *Blumea lacera*          | Compositae      | Kukursunga  | NA         | L: astringent, stimulant, antihelminthic, antimicrobial, anti-inflammatory and diuretic |
| *Bruguiera gymnorrhiza*  | Rhizophoraceae  | Kankra      | DACB 31386  | B: astringent, diarrhoea, stops bleeding; L: blood pressure |
| *Clerodendron inerme*    | Verbenaceae     | Bon Jui     | DACB 31537  | AP: hypotensive, fever; R: rheumatism, cancer prevention (India) |
| *Cynometra ramiflora*    | Liguminosae     | Kucha       | NA         | None reported                                         |
| *Ficus religiosa*        | Moraceae        | Pan Bot     | DACB 32004  | B: antibacterial, astringent, diarrhoea, dysentery, gonorrhoea, antiprotozoal, antiviral and ulcers; L: skin disease |
| *Hibiscus tiliaceus*     | Malvaceae       | Bhola       | DACB 31539  | L: fever, coughs and dry throat; F: bronchitis, ear infections, dysentery, chest congestion |
| *Hygrophila auriculata*  | Acanthaceae     | Talmakna    | DACB 31257  | S: tonic, diarrhoea, dysentery, urinary discharge, gonorrhoea, diuretic, hepatoprotective; L: inflammation, rheumatism; AP: antineoplastic |
| *Limnaphila indica*      | Scrophulariaceae| Karpur      | DACB 31536  | AP: antiseptic, with coconut oil is used in elephantiasis, fever; WP: dysentery |
| *Mollugo pentaphylla*    | Molluginaceae   | Khetpapra   | NA         | L: antiseptic, used in digestion, relieve ear ache, spermicidal and antifungal |
| *Pandanus foetidus*      | Pandanaceae     | Kewa kata   | DACB 31541  | WP: leprosy, small pox, syphilis, scabies and heart and brain diseases; L: spadix and diabetes |
| *Xylocarpus moluccensis* | Meliaceae       | Passur      | DACB 31540  | B: astringent, febrifuge, dysentery, diarrhoea; F: cure for elephantiasis and swelling of the breasts; S: itch |

NA: not available; AP: aerial parts; B: bark; F: flowers; L: leaves; R: roots; S: seeds; WP: whole plant.

or antibacterial activity (*Adiantum caudatum*, *F. religiosa*, *M. pentaphylla* and *Argemone mexicana*) [17, 23–25] has been reported.

The majority of plant-based natural products are pheno- lolic compounds [26]. Anticancer activity has been shown to be associated with a variety of classes, such as polyphenols, flavonoids and catechins [27]. A number of flavonoids and polyphenols have previously been isolated from different parts of *Hygrophila auriculata*, *L. indica*, *Bruguiera gymnorrhiza*, *Clerodendron inerme*, *Blumea lacera*, *Hibiscus tiliaceus*, *X. moluccensis* and *Aegiceras corniculatum* [5, 22, 28–36], which may be involved in their reported cytotoxic activity. Interestingly, no alkaloids, lectins or polysaccharides have been isolated to date from these plants, except an alkaloid from *Argemone mexicana* [37]. Here we report for the first time on the cytotoxic activity of methanolic and aqueous extracts from 16 Bangladeshi medicinal plants against normal mouse fibroblasts (NIH3T3), gastric cancer (AGS), colon cancer (HT29) and breast cancer (MDA-MB-435S) cells.

2. Methods

2.1. Plant Material. From March 2006 to May 2007, 16 plants were collected from tidal forests in the coastal Sundarbans (a swamp region in the Ganges delta), and other locations in the Khulna district of Bangladesh. The plant material was identified by the Bangladesh National Herbarium, Dhaka, Bangladesh and shade-dried. A specimen representing each collection was deposited in the Bangladesh National Herbarium, Dhaka, Bangladesh (Table 1).

2.2. Chemicals. *n*-Hexane, dichloromethane and methanol were purchased from Merck, Germany. Advanced Dulbecco's
modified Eagle’s medium (DMEM) (Batch #497466 and ID: Gibco 12491), newborn calf serum (NBCS) (Batch #1280182 and ID: Gibco 2901), trypsin-EDTA (Batch #475919 and ID: Gibco 25200) and L-glutamine (Batch #371023 and ID: Gibco 25300) were all obtained from Invitrogen, Australia. Dimethylsulfoxide (DMSO) (Batch #038K07101 and ID: Sigma D8418-100 mL), [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) (Batch #02317KH and ID: Sigma M2128-1G) were supplied from Sigma Aldrich, Germany.

2.3. Preparation of Extracts. The dried plant material (50–200 g) was ground into coarse powder and then successively extracted with solvents of decreasing lipophilicity (n-hexane, dichloromethane, methanol and milliQ-water) using a Soxhlet apparatus. The plant extracts were then filtered and the solvent was evaporated under reduced pressure followed by freeze-drying.

2.4. Cytotoxic Screening

2.4.1. Cell Culture. Normal mouse fibroblast cells (NIH/3T3, ATCC: CRL-1658) and three human cancer cell lines gastric adenocarcinoma cells (AGS, ATCC: CRL-1739), colorectal adenocarcinoma cells (HT-29, ATCC: HTB-38) and breast ductal carcinoma cells (MDA-MB-435S, ATCC: HTB-129) were used for cytotoxicity screening of the Bangladeshi medicinal plant extracts. All cell lines were purchased from ATCC, Manassas, VA 20108, USA. Cell lines were cultured in Advanced DMEM supplemented with 10% inactivated NBCS and 5 mM L-glutamine, and grown at 37°C in a humidified atmosphere of 5% CO₂ in air.

2.5. MTT Assay. The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay developed by Mosmann [38], and further modified by Popiolkiewicz [39] and Kim [40], was used with minor modifications to screen for cytotoxic activity of Bangladeshi medicinal plant extracts. Briefly, the cells were seeded in 96-well plates at a density of 2.5 × 10⁴ cells/well. Following 24-h incubation and attachment, the cells were treated with different concentrations of plant extract for 24 h. Following washing and incubation with MTT solution (0.5 mg mL⁻¹ for cancer cell lines and 1 mg mL⁻¹ for mouse fibroblasts) for 2 h, the cells were lysed with DMSO. The absorbance was measured after 45 min using a microplate reader (Wallac 1420 Multilabel counter, PerkinElmer) at a wavelength of 560 nm. MilliQ-water and 0.75% DMSO served as the negative control for water and methanol extracts, respectively, while 25% DMSO served as the positive control. The MTT assay was validated using various concentrations of DMSO (0.25–25%).

Extracts showing cytotoxic activity were further tested at additional concentrations to calculate the IC₅₀ values. The results are generated from two independent experiments; each experiment was performed in triplicate. The IC₅₀ values were calculated with probit analysis software (LdP Line software, USA).

3. Results

A total of 32 extracts representing 16 Bangladeshi plant species from 16 plant families were screened for their cytotoxic activity against healthy mouse fibroblast and three human cancer cell lines (gastric, colon and breast cancer cells). The cytotoxic activities of the methanolic and aqueous extracts of the plants are summarized in Table 2. It is worth noting that IC₅₀ values between 1 and 2 mg mL⁻¹, while somewhat high, still point subtly towards selective activity. These “high” values are likely due to very low concentrations of compounds of interest, which would be considerably enriched upon bioactivity-guided fractionation.

3.1. Selective Cancer-Cell Cytotoxic Activity. Importantly, among the 32 extracts tested, three extracts, namely the methanolic extract of Hygrophila auriculata and Hibiscus tiliaceous, as well as the aqueous extract of L. indica showed no evident cytotoxicity against healthy mouse fibroblast cells, but selective cytotoxicity, particularly against breast cancer cells (IC₅₀ 1.1–1.6 mg mL⁻¹). Seven methanolic extracts (Acrostichum aureum, Argemone mexicana, Ammannia baccifera, Clerodendron inerme, Cynometra ramiflora, L. indica, X. moluccensis) and four aqueous extracts (Aegiceras corniculatum, Bruguiera gymnorrhiza, Hygrophila auriculata, X. moluccensis) showed low toxicity (IC₅₀ < 2.5 mg mL⁻¹) against mouse fibroblasts but selective cytotoxicity against different cancer cell lines. For example, the methanol extract from Ammannia baccifera leaves displayed selective cancer cell line cytotoxicity with IC₅₀ values of 0.55, 0.59 and 0.91 mg mL⁻¹ against gastric, colon and breast cancer cells, respectively. Similarly, the methanol extract from the pneumatophore of X. moluccensis showed IC₅₀ values of 0.62 and 1.08 mg mL⁻¹ against gastric and breast cancer cells, respectively. Moreover, the aqueous seed extract from Hygrophila auriculata displayed selective cancer cell cytotoxicity with an IC₅₀ value of 0.22 mg mL⁻¹ against colon cancer cells.

3.2. High Non-Selective Cytotoxic Activity. Four extracts showed cytotoxic activity against all tested cell lines including the healthy cell line. The methanolic extract of Adiantum caudatum leaves displayed moderate cytotoxicity (IC₅₀ 1.23–1.88 mg mL⁻¹), whereas the aqueous extract from Hibiscus tiliaceous leaves showed significantly lower IC₅₀ values, especially against gastric (IC₅₀ 0.25 mg mL⁻¹) and colon cancer cells (IC₅₀ 0.8 mg mL⁻¹). However, the methanolic extract from Blumea lacera leaves showed the highest cytotoxicity (IC₅₀ 0.01–0.08 mg mL⁻¹) against all tested cell lines among all extracts tested in this study.

3.3. Low or No Cytotoxic Activity. It should also be noted that 9 of the 16 aqueous Bangladeshi plant extracts showed no or very low cytotoxic activity against healthy or cancer cell lines tested, whereas this is the case for only 3 of the 16 methanolic extracts. The low cytotoxic potential of the aqueous extracts is of great significance for their traditional use in the treatment of various disorders other than cancer.
Table 2: Cytotoxic activity (IC\(_{50}\)) of Bangladeshi plant extracts.

| Species name                | Part used | Extract | Yields (%) | NIH/3T3 | AGS | HT29 | MDA-MB-435S |
|----------------------------|-----------|---------|------------|---------|-----|------|-------------|
| Acrostichum aureum         | L         | M       | 0.64       | >2.50   | 1.02| >2.50| >2.50       |
|                           | W         |         | 2.94       | >2.50   | >2.50| >2.50| >2.50       |
| Adiantum caudatum          | L         | M       | 11.14      | 1.88    | 1.75| 1.48 | 1.23        |
|                           | W         |         | 8.04       | >2.50   | >2.50| >2.50| >2.50       |
| Aegiceras corniculatum     | B         | M       | 6.39       | 0.02    | >2.50| 0.33 | 0.66        |
|                           | W         |         | 1.40       | >2.50   | 1.68 | NC   | 1.91        |
| Argemone mexicana          | L         | M       | 5.28       | >2.50   | >2.50| >2.50| >2.50       |
|                           | W         |         | 10.90      | >2.50   | >2.50| >2.50| >2.50       |
| Ammannia baccifera         | L         | M       | 20.16      | >2.50   | 0.55 | 0.59 | 0.91        |
|                           | W         |         | 4.25       | >2.50   | >2.50| >2.50| >2.50       |
| Blumea lacera              | L         | M       | 15.41      | 0.01    | 0.03 | 0.07 | 0.08        |
|                           | W         |         | 16.15      | 0.67    | 0.99 | 0.48 | 0.39        |
| Bruguiera gymnorrhiza      | L         | M       | 4.42       | NC      | >2.50| >2.50| >2.50       |
|                           | W         |         | 2.15       | >2.50   | >2.50| >2.50| 1.38        |
| Clerodendron inerme        | L         | M       | 1.43       | >2.50   | 2.38 | >2.50| >2.50       |
|                           | W         |         | 3.24       | >2.50   | >2.50| >2.50| >2.50       |
| Cynometra ramiflora        | B         | M       | 6.16       | >2.50   | >2.50| 1.79 | 2.35        |
|                           | W         |         | 2.91       | >2.50   | >2.50| >2.50| >2.50       |
| Ficus religiosa            | L         | M       | 1.14       | 1.01    | 2.16 | >2.50| >2.50       |
|                           | W         |         | 1.33       | >2.50   | NC   | >2.50| >2.50       |
| Hibiscus tiliaceous        | L         | M       | 4.84       | NC      | 2.50 | >2.50| 1.14        |
|                           | W         |         | 5.94       | 1.11    | 0.25 | 0.80 | 1.09        |
| Hygrophila auriculata      | S         | M       | 0.51       | NC      | >2.50| >2.50| 1.58        |
|                           | W         |         | 4.36       | >2.50   | >2.50| 0.22 | 1.40        |
| Limnophila indica          | L         | M       | 11.14      | >2.50   | >2.50| 2.19 | 1.24        |
|                           | W         |         | 5.04       | NC      | 2.24 | NC   | 1.25        |
| Mollugo pentaphylla        | WP        | M       | 6.22       | >2.50   | >2.50| >2.50| >2.50       |
|                           | W         |         | 3.97       | >2.50   | >2.50| NC   | >2.50       |
| Pandanus foetidus          | L         | M       | 5.70       | >2.50   | NC   | >2.50| >2.50       |
|                           | W         |         | 4.45       | >2.50   | >2.50| >2.50| >2.50       |
| Xylocarpus moluccensis     | P         | M       | 20.07      | >2.50   | 0.62 | >2.50| 1.08        |
|                           | W         |         | 5.42       | >2.50   | >2.50| >2.50| 1.78        |

B: bark; L: leaves; S: seeds; WP: whole plant; P: pneumatophore; M: methanolic extract; W: aqueous extract; *NC: no cytotoxicity at a concentration up to 2.5 mg mL\(^{-1}\); IC\(_{50}\) (50% inhibition of cell growth) calculated by probit analysis software, data was generated from two independent experiments, each experiment performed in triplicates.

4. Discussion

Complementary and alternative medicine (CAM) reports on multiple holistic approaches, including herbal medicines [41]. Recently, CAM has directed its interest towards therapies focused on important diseases throughout the world [42]. Drug discovery from natural sources is an area pertinent to CAM [43] and natural sources such as plants, animals and microorganisms provide a basis for the isolation of unique and potentially potent bioactive compounds [44]. Ethnopharmacologists can therefore provide CAM practitioners with relevant new information on therapies from natural sources [44]. This information helps to establish modern CAM treatment modalities, which may offer efficacious treatment to large populations affected with different diseases including cancer [45]. For example, a few studies into the anticancer potential of plants used in Bangladeshi folk medicine have been performed [46, 47].

Our study describes investigations into the anticancer potential of 16 so far not studied Bangladeshi medicinal plants by screening for cytotoxic activity against healthy mouse fibroblasts and three human cancer cell lines. Some plant extracts showed low or no toxicity against healthy mouse fibroblasts, but selective cytotoxicity against breast cancer cells, whereas others showed high cytotoxicity against all cell lines or were not cytotoxic against any of the cell lines tested.

Among the plant extracts that showed low toxicity against mouse fibroblasts but selective cytotoxicity against...
different cancer cell lines, the methanolic extract of *Acrostichum aureum* leaves showed the most potent selective cytotoxicity. Interestingly, in one study cytotoxic activity against HeLa cells has been reported for *Acrostichum aureum* [48].

The aqueous extracts from the seeds *Hygrophila auriculata*, with low toxicity against mouse fibroblasts and selective cytotoxicity against different cancer cell lines, has been previously been used as a traditional anticancer treatment [5]. Moreover, other in vitro studies have reported antioxidant (aerial parts), hepatoprotective (aqueous root extract), antitumor (petroleum ether root extract) and NFkB inhibition [9, 49] for extracts of *Hygrophila auriculata*.

In contrast, *Clerodendron inerme* is used for cancer prevention in the Indian traditional medical system [13]. Also, extracts of *Clerodendron inerme* have reported cytotoxic activity on oral squamous cells from 7,12-dimethylbenz[a]anthracene (DMBA) induced carcinogenesis [13]. Not surprisingly therefore, our study did not detect any significant cytotoxic effects of extracts from *Clerodendron inerme*.

The methanolic extracts from *Adiantum caudatum* and *Blumea lacera*, along with the aqueous extracts from *Blumea lacera* and *Hibiscus tiliaceous* showed high cytotoxicity against all cell lines tested, with the methanolic extract of *Blumea lacera* being the most cytotoxic amongst all tested plant extracts. Neither of these plants have previously been used as anticancer treatments in traditional Bangladeshi medicine, although, a hot aqueous extract of *Blumea lacera* has been reported to elicit cytotoxic activity against K562 cells (Human erythromyeloblastoid leukemia cells) [7].

The methanolic extract of *Aegiceras corniculatum* showed very high cytotoxic activity against healthy, colon and breast cancer cells with IC$_{50}$ values ranging from 0.02 to 0.66 mg mL$^{-1}$, but very low cytotoxicity against gastric cancer cells. Interestingly, the plant *Aegiceras corniculatum* has been used traditionally as fish poison [32], however, no anticancer or cytotoxic activities have been reported to date.

Methanolic extracts of *Bruguiera gymnorrhiza* reported cytoprotective activity on bovine aortal endothelial cells (BAEC) against oxidized Low Density Lipoprotein (LDL) induced cytotoxicity [11]. Moreover, the petroleum ether extract (flowers) of *Bruguiera gymnorrhiza* has shown inhibitory activity for NFk-B and COX-2 [50]. In our study, aqueous and methanol extracts of *Bruguiera gymnorrhiza* leaves showed low to no cytotoxicity against any cell line, apart from the water extract, which displayed moderate selective cytotoxicity (IC$_{50}$ 1.38 mg mL$^{-1}$) against breast ductal carcinoma cells (MDA-MB-453S).

*XYLOCARPUS MOLUCCENSIS* has been used traditionally in the treatment of swollen breasts [5]. More specific information is unfortunately unavailable; however, swollen breasts are usually a consequence of, hormonal changes, inflammation or benign or cancerous growth. Although it may not be directly related, it is interesting to note that in our study both extracts of *X. moluccensis* displayed moderate cytotoxic activity against breast ductal carcinoma cells.

This is the first time that aqueous and methanolic extracts from the 16 listed Bangladeshi plants (*Acrostichum aureum*, *Adiantum caudatum*, *Aegiceras corniculatum*, *Ammannia baccifera*, *Argemone mexicana*, *Blumea lacera*, *Bruguiera gymnorrhiza*, *Clerodendron inerme*, *Cynometra latifolia*, *Cynometra ramiflora*, *F. religiosa*, *Hibiscus tiliaceous*, *Hygrophila auriculata*, *L. indica*, *P. foetida*, and *X. moluccensis*) have been screened against human gastric, colon and breast cancer cell lines. This study supports the traditional uses of *Hygrophila auriculata*, *Clerodendron inerme*, and the reported cytotoxic activities of *Blumea lacera*, *Argemone mexicana* and *Acrostichum aureum*. Some of the plant extracts, such as *L. indica*, *Hibiscus tiliaceous*, *Cynometra ramiflora*, *Ammannia baccifera* and *Adiantum caudatum*, exerted selective cytotoxic activity, but neither cytotoxic activity had been reported previously, nor were the plants used traditionally in the treatment of cancer. This study provides an important basis for further investigation into the isolation, characterisation and mechanism of cytotoxic compounds from some of the screened Bangladeshi medicinal plants. Thus, these plants could be used as a source for new lead structures in drug design to combat cancer.

**Acknowledgments**

The authors would like to thank Griffith University, Australia, for providing financial support in the form of scholarships to Shaikh Jamal Uddin.

**References**

[1] D. J. Newman and G. M. Cragg, “Natural products as sources of new drugs over the last 25 years,” *Journal of Natural Products*, vol. 70, no. 3, pp. 461–477, 2007.

[2] S. M. K. Rates, “Plants as source of drugs,” *Toxicol*, vol. 39, no. 5, pp. 603–613, 2001.

[3] J. G. Graham, M. L. Quinn, D. S. Fabricant, and N. R. Farnsworth, “Plants used against cancer—an extension of the work of Jonathan Hartwell,” *Journal of Ethnopharmacology*, vol. 73, no. 3, pp. 347–377, 2000.

[4] FAO, *Trade in Medicinal Plants*, Economic and Social Department, Food and Agriculture Organization of the United Nations, Rome, Italy, 2004.

[5] A. Ghani, *Medicinal Plants of Bangladesh with Chemical Constituents and Uses*, Asiatic Society of Bangladesh, Dhaka, Bangladesh, 2003.

[6] M. Yusuf, J. U. Chowdhury, M. A. Wahab, and J. Begum, *Medicinal Plants of Bangladesh*, Bangladesh Council of Scientific & Industrial Research (BCSIR), Dhaka, Bangladesh, 1994.

[7] L.-C. Chiang, H.-Y. Cheng, C.-C. Chen, and C.-C. Lin, “In vitro anti-leukemic and antiviral activities of traditionally used medicinal plants in Taiwan,” *American Journal of Chinese Medicine*, vol. 32, no. 5, pp. 695–704, 2004.

[8] E. Lambertini, R. Piva, M. T. Khan et al., “Effects of extracts from Bangladeshi medicinal plants on in vitro proliferation of human breast cancer cell lines and expression of estrogen receptor alpha gene,” *International Journal of Oncology*, vol. 24, pp. 419–423, 2004.
Evidence-Based Complementary and Alternative Medicine

[9] I. Lampronti, M. T. Khan, N. Bianchi et al., “Bangladeshi medicinal plant extracts inhibiting molecular interactions between nuclear factors and target DNA sequences mimicking NF-kappaB binding sites,” Journal of Medicinal Chemistry, vol. 1, pp. 327–333, 2005.

[10] J.-J. Chen, S.-Y. Huang, C.-Y. Duh, I.-S. Chen, T.-C. Wang, and H.-Y. Fang, “A new cytotoxic amide from the stem wood of Hibiscus tiliaceus,” Planta Medica, vol. 72, no. 10, pp. 935–938, 2006.

[11] P. L. Owen, T. Matainaho, M. Sirois, and T. Johns, “Endothelial cyttoprotection from oxidized LDL by some crude Melanesian plant extracts is not related to their antioxidant capacity,” Journal of Biochemical and Molecular Toxicology, vol. 21, pp. 231–242, 2007.

[12] R. M. Rosa, D. I. Moura, M. I. S. Melechci et al., “Protective effects of Hibiscus tiliaceus L. methanolic extract to V79 cells against cytotoxicity and genotoxicity induced by hydrogen peroxide and tert-butyl-hydroperoxide,” Toxicology in Vitro, vol. 21, no. 8, pp. 1442–1452, 2007.

[13] S. Manoharan, K. Kavitha, S. Balakrishnan, and K. Rajalingam, “Clerodendrum inerme protects cellular integrity during 7,12-dimethylbenz [A]-anthracene induced hamster buccal pouch carcinogenesis,” African Journal of Traditional, Complementary and Alternative Medicines, vol. 5, no. 2, pp. 213–222, 2008.

[14] B. Ye, Y. Zheng, M. Wang, W. Li, X. Hu, and Y. Wang, Isolation and Identification of New Flavonoid from Bruguiera gymnorhiza and Antitumor Application Therof, China, 2008.

[15] I. Lampronti, M. T. H. Khan, M. Borgatti, N. Bianchi, and R. Gambari, “Inhibitory effects of Bangladeshi medicinal plant extracts on interactions between transcription factors and target DNA sequences,” Evidence-Based Complementary and Alternative Medicine, vol. 5, no. 3, pp. 303–312, 2008.

[16] T. Roome, A. Dar, S. Ali, S. Naqvi, and M. I. Choudhary, “A study on antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective actions of Aegiceras corniculatum (stem) extracts,” Journal of Ethnopharmacology, vol. 118, no. 3, pp. 514–521, 2008.

[17] S.-S. Kim, J.-Y. Kim, N. H. Lee, and C.-G. Hyun, “Antibacterial and anti-inflammatory effects of Jeju medicinal plants against acne-inducing bacteria,” Journal of General and Applied Microbiology, vol. 54, no. 2, pp. 101–106, 2008.

[18] S. Somasundaram and J. Sadique, “The role of mitochondrial calcium transport during inflammation and the effect of anti-inflammatory drugs,” Biochemical Medicine and Metabolic Biology, vol. 36, no. 2, pp. 220–230, 1986.

[19] R. M. Rosa, M. I. Melecchi, R. da Costa Halmenschlager, F. C. Abad, C. R. Simon, E. B. Caramao et al., “Antioxidant and antimutagenic properties of Hibiscus tiliaceus L. methanolic extract,” Journal of Agricultural and Food Chemistry, vol. 54, pp. 7324–7330, 2006.

[20] M. Vijayakumar, R. Govindarajan, G. M. M. Rao et al., “Action of Hygrophila auriculata against streptozotocin-induced oxidative stress,” Journal of Ethnopharmacology, vol. 104, no. 3, pp. 356–361, 2006.

[21] D. Banerjee, S. Chakrabarti, A. K. Hazra, S. Banerjee, J. Ray, and B. Mukherjee, “Antioxidant activity and total phenolics of some mangroves in Sundarbans,” African Journal of Biotechnology, vol. 7, no. 6, pp. 805–810, 2008.

[22] S. J. Uddin, J. A. Shilpi, S. M. Alam, M. Alamgir, M. T. Rahman, and S. D.arker, “Antidiarrhoeal activity of the methanol extract of the barks of Xylocarpus moluccensis in castor oil- and magnesium sulphate-induced diarrhoea models in mice,” Journal of Ethnopharmacology, vol. 101, pp. 139–143, 2005.

[23] I. Bhattacharjee, S. K. Chatterjee, S. Chatterjee, and G. Chandra, “Antibacterial potentiality of Argemone mexicana solvent extracts against some pathogenic bacteria,” Memorias do Instituto Oswaldo Cruz, vol. 101, no. 6, pp. 645–648, 2006.

[24] M. Singh, N. Singh, P. B. Khare, and A. K. S. Rawat, “Antimicrobial activity of some important Adiantum species used traditionally in indigenous systems of medicine,” Journal of Ethnopharmacology, vol. 115, no. 2, pp. 327–329, 2008.

[25] J. B. McAlpine, W. A. Chu, S. Ratnayake, J. B. Jiang, A. M. Stafford, and C. Jackson, in Antimicrobial Auran Derivatives, P. I. Appl., Ed., p. 47, 1997.

[26] A. Kirakosyan, I. A. Duke, P. B. Kaufman, S. Warber, and L. J. Ceeke, Natural Products from Plants, Taylor & Francis, New York, NY, USA, 2006.

[27] K. D. Park, S. G. Lee, S. U. Kim et al., “Anticancer activity of 3-O-acetyl and alkyl(-)epicatechin derivatives,” Bioorganic and Medicinal Chemistry Letters, vol. 14, pp. 5189–5192, 2004.

[28] R. C. Bheemasankara, R. T. Namosiva, and B. Muralikrishna, “Flavonoid from Blumea lacera,” Planta Medica, vol. 31, no. 3, pp. 235–237, 1977.

[29] A. Ghosh, S. Misra, A. K. Dutta, and A. Choudhury, “Pentacyclic triterpenoids and sterols from seven species of mangrove,” Phytochemistry, vol. 24, no. 8, pp. 1725–1727, 1985.

[30] I. Laakso, T. Seppanen-Laakso, R. Hiltunen, and O. Ekundayo, “Composition of the essential oil fo Blumea lacera DC. (Asteraceae) leaves from Nigeria,” Flavour and Fragrance Journal, vol. 4, pp. 73–75, 1989.

[31] R. Agarwal, R. Singh, I. R. Siddiqui, and J. Singh, “Triterpenoid and prenylated phenol glycosides from Blumea lacera,” Phytochemistry, vol. 38, no. 4, pp. 935–938, 1995.

[32] W. M. Bandaranayake, “Bioactivities, bioactive compounds and chemical constituents of mangrove plants,” Wetlands Ecology and Management, vol. 10, no. 6, pp. 421–452, 2002.

[33] R. Pandey, R. K. Verma, and M. M. Gupta, “Neo-clerodane diterpenoids from Clerodendrum inerme,” Phytochemistry, vol. 66, no. 6, pp. 643–648, 2005.

[34] D. Zhang, S. Zhang, J. Wu, J. Huang, Y. Tian, and L. Xu, “Pentacyclic triterpenes from Aegiceras corniculatum,” Tianran Chanwu Yanjiu Yu Kaifa, vol. 17, pp. 306–308, 2005.

[35] N. P. Reddy, B. A. K. Reddy, D. Gunasekar, A. Blond, B. Bodo, and M. M. Murthy, “Flavonoids from Limnophila indica,” Phytochemistry, vol. 68, no. 5, pp. 636–639, 2007.

[36] C. Feng, X.-M. Li, N.-Y. Ji, and B.-G. Wang, “Triterpenoids from the mangrove plant Hibiscus tiliaceus,” Helvatica Chimica Acta, vol. 91, no. 5, pp. 850–855, 2008.

[37] Y.-C. Chang, F.-R. Chang, A. T. Khalil, P.-W. Hsieh, and Y.-C. Wu, “Cytoxic benzophenanthridine and benzylisoquinoline alkaloids from Argemone mexicana,” Zeitschrift fur Naturforsch C, vol. 58, no. 7–8, pp. 521–526, 2003.

[38] T. Mosmann, “Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays,” Journal of Immunological Methods, vol. 65, no. 1-2, pp. 55–63, 1983.

[39] J. Popiłówkiewicz, K. Polkowski, J. S. Skierski, and A. P. Mazurek, “In vitro toxicity evaluation in the development of new anticancer drugs—genistein glycosides,” Cancer Letters, vol. 229, no. 1, pp. 67–75, 2005.
[40] S. C. Kim, S. J. Park, J. R. Lee, J. C. Seo, C. H. Yang, and S. H. Byun, "Cytoprotective activity of Glycyrrhizae radix extract against arsenite-induced cytotoxicity," Evidence-Based Complementary and Alternative Medicine, vol. 5, no. 2, pp. 165–171, 2008.

[41] E. L. Cooper, "Stem cells and CAM," Evidence-Based Complementary and Alternative Medicine, vol. 3, pp. 167–169, 2006.

[42] B. Saad, H. Azaiz, and O. Said, "Tradition and perspectives of Arab herbal medicine: a review," Evidence-Based Complementary and Alternative Medicine, vol. 2, no. 4, pp. 475–479, 2005.

[43] E. L. Cooper, "Drug discovery, CAM and natural products," Evidence-Based Complementary and Alternative Medicine, vol. 1, pp. 215–217, 2004.

[44] E. L. Cooper, "eCAM: an emerging linkage with ethnopharmacology?" Evidence-Based Complementary and Alternative Medicine, vol. 5, no. 4, pp. 365–366, 2008.

[45] A. Vojdani and J. Erde, "Regulatory T cells, a potent immunoregulatory target for CAM researchers: modulating tumor immunity, autoimmunity and alloreactive immunity (III)," Evidence-Based Complementary and Alternative Medicine, vol. 3, no. 3, pp. 309–316, 2006.

[46] L. V. Costa-Lotufo, M. T. H. Khan, A. Ather et al., "Studies of the anticancer potential of plants used in Bangladeshi folk medicine," Journal of Ethnopharmacology, vol. 99, no. 1, pp. 21–30, 2005.

[47] I. Lampronti, D. Martello, N. Bianchi et al., "In vitro antiproliferative effects on human tumor cell lines of extracts from the Bangladeshi medicinal plant Aegle marmelos Correa," Phytomedicine, vol. 10, no. 4, pp. 300–308, 2003.

[48] H. Dai, W. Mei, K. Hong, Y. Zeng, and L. Zhuang, "Screening of the tumor cytotoxic activity of sixteen species of mangrove plants in Hainan," Zhongguo Haiyang Yaowu, vol. 24, pp. 44–46, 2005.

[49] P. Shanmugasundaram and S. Venkataraman, "Hepatoprotective and antioxidant effects of Hygrophila auriculata (K. Schum) Heine Acanthaceae root extract," Journal of Ethnopharmacology, vol. 104, no. 1-2, pp. 124–128, 2006.

[50] S. Homhual, N. Bunyapraphatsara, T. Kondratyuk et al., "Bioactive dammarane triterpenes from the mangrove plant Bruguiera gymnorrhiza," Journal of Natural Products, vol. 69, no. 3, pp. 421–424, 2006.