Original Research Article

Screening of fifteen mangrove plants found in Sri Lanka for *in-vitro* cytotoxic properties in breast (MCF-7) and hepatocellular carcinoma (HepG2) cells.

ABSTRACT

Aims:

Evaluation of cytotoxic potential of leaf and stem bark extracts of 15 mangrove plants grown in Sri Lanka in breast cancer (MCF -7) and hepatocellular (Hep G2) carcinoma cells grown in Sri Lanka.

Place and Duration of Study:

At the Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo between 1st 14 of February 2014 to April 2015.

Methodology:

Leaves and stem barks of 15 mangrove plants were extracted with hexane, chloroform, ethyl acetate and methanol. Resulting extracts were screened for cytotoxic activity against MCF -7 and HepG2 cells using the Sulforhodamine B (SRB) assay.

Results:

Of the 15 plants tested, *Phoenix paludosa, Avicennia officinaliss* and *Scyphiphora hydrophyllacea* showed highest cytotoxic properties in cancer cells. Chloroform extract of stem bark of *S. hydrophyllacea*, *Bruguiera gymnorrhiza* (chloroform, ethyl acetate and methanol extracts of leaves), hexane and ethyl acetate extracts of leaves of *Aegiceras corniculatum*, methanol extracts of leaves and stem bark of Nypa *fruticans* and *Rhizophora mucronata,* methanol extract of stem bark of *Sonneratia alaba* and *Rhizophora apiculata* and methanol extract of bark of *A. officinails* exerted selective cytotoxicity to HepG2 cells. The hexane extract of leaves of *B. gymnorrhiza*, chloroform extract of leaves of *N. fruticans*, ethyl acetate extract of stem bark of *Lumnitzera littorea*, chloroform extract of leaves of *Rhizophora apiculata* and chloroform extract of leaves of *Pemphis acidula* showed selective cytotoxic effects against MCF-7 cells. Out of the 120 mangrove extracts tested, 82 extracts showed no significant cytotoxic effects (IC_{50}>100 µg/mL) against MCF 7 or Hep G2 cells.

Conclusion:

The cytotoxic activities demonstrated by some of the solvent extracts of some mangrove plants provide scientific evidence for their therapeutic potentials and further studies are needed to identify active compounds responsible for cytotoxic effects.

Keywords: *Mangrove, cytotoxicity, MCF-7, HepG2*

1. Introduction

Mangroves belong to twelve plant families and they are botanically diverse. Almost all the mangroves are holophytic species, well adapted to grow in wet soil conditions and usually possess some amount of viviparity [1, 2]. The mangroves grown in Sri Lanka belong to true mangroves (14 species) and mangrove associates (12 species) [3]. In Sri Lanka mangroves are extensively found in Puttalam,

Kalpitiya, Koggala, Kalamatiya and Kokilai areas in association with estuaries [46 4]. Mangroves have diverse uses: For example, they are used to obtain timber and tannins; they behave as coastal stabilizers; root system in mangroves provides shelter for many commercially important fishes and prawns, etc. [5]. These mangrove plants can survive in extremely high salinity, high temperature, high

moisture, strong winds and high and low tides of water. In order to survive in these hostile environments, changes in their physiological activities have occurred ensuing in the bio-synthesis of novel secondary metabolites [6]. These secondary metabolites provide proper protection to these mangrove plants against various biotic and abiotic stresses conditions [7]. A wide range of natural compounds, including novel chemical compounds have been isolated from mangroves and mangrove associates. Alkaloids, alcohols, amino acids, fatty acids, lipids, phenolic compounds, steroids, tannins, flavonoids, halogenated compounds, pheromones, phorbol esters and triterpins are among these isolated compounds [8, 9]. Some isolated compounds from mangroves are considered to have bioactivities that may be beneficial to improve human health and these compounds might be very useful in new drug discovery process [10]. Mangrove plants have also been used as a folklore medicine and extracts from mangroves have been reported to have biological activities including cytotoxic, anti-bacterial, anti-viral and anti-inflammatory, etc as shown in Table 1. However, there is no data available on *in-vitro* cytotoxic properties of leaves and stem bark of most of the mangroves grown in Sri Lanka in breast (MCF -7) and hepatocellular carcinoma (HepG2) cells. Therefore, the main aim of this study was to evaluate the cytotoxic potential of leaves and stem bark of 15 selected mangroves/mangrove associates grown in Sri Lanka by evaluating of their effects in breast (MCF-7) and hepatocellular carcinoma (HepG2) cells.

2. Materials and methods

2.1. Chemicals

Fetal bovine serum (FBS), trypsin-EDTA, strep-penicillin, HepG2 cells, MCF-7 cells and Dulbecco's modified Eagle's medium (DMEM) were purchased from American Type Culture Collection (ATCC),

USA. All the chemicals used in the study were purchased from Sigma-Aldrich (77 St Louis, MO, USA) unless otherwise specified.

2.2 Plant material

Healthy leaves and barks of 15 selected mangrove plants were collected from the mangrove park, Kadolkele, Negombo in the Western Province of Sri Lanka and Kalpitiya area in the North Western Province of Sri Lanka. Plants were identified by the Botanists at the National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka and by Mr. W.A. Sumanadasa, of the National Aquatic Resources Research and Development Agency (*NARA*), Negombo. Voucher specimens were deposited in the National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka and Institute of Biochemistry Molecular Biology and Biochemistry, University of Colombo Sri Lanka (Table 2).

2.3 Preparation of plant extracts

Collected mangrove leaves and barks were dried at room temperature for 4-7 days and ground into powder. Ground leaf and bark samples (10 g each) were extracted sequentially in to hexane, chloroform, ethyl acetate and methanol respectively by sonication at room temperature. All the resulting extracts (sixty leaf extracts and fifty six stem bark extracts) were filtered and concentrated under vacuum in a rotary evaporator (Rotavapor® R-/ BUCHI, Switzerland). Stock solutions were prepared by dissolving all extracts in dimethyl sulfoxide (DMSO).

2.4 Cell culture maintenance

HepG2 and MCF-7 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum and 50 IU/mL penicillin and 50 µg/mL streptomycin at 37 ºC in a humidified environment (95 % air ;5 % CO_2). At 80 % confluency, cancer cells were trypsinized and seeded ($5x10_3$ cells/well) in 96-well cell culture plates and incubated for 24 h.

2.5 Assessment of cytotoxicity

After 24 h incubation, cells were exposed to leaf and bark extracts (doses ranging from 25 to 400 µg/mL and in triplicates) of mangroves for 24 and 48 h and cytotoxicity assessed by Sulforhodamine B assay (SRB) as previously described by us [61, 62]. Briefly, treated cells were washed three times with PBS and fixed with Trichloroacetic acid (10%). Fixed cells were washed with tap water five times and then SRB (0.4 %) was added to each well and incubated for 20 min. Unbound dye was removed by washing with acetic acid and bound dye was solubilized with Tris base (10 mM; pH 7.5). Plates were then kept on a plate shaker for 1 h and absorbance was taken at 540 nm using Synergy™ HT Multi-Mode Microplate Reader (BioTek, USA).

2.6 Statistical analysis

All the experiments in this study were carried out at least three times in triplicate. Data were analyzed using Prism 5.0 (Graph pad Prism) statistical software package and results were expressed as mean ± standard deviation (SD).

3. Results

A total of 116 solvent extracts (hexane, chloroform, ethyl acetate and methanol extracts of leaf and stem bark) representing 15 mangrove/mangrove associates and mangrove minors collected from Sri Lanka were tested for their cytotoxic effects on MCF 7 and HepG2 cells. The cytotoxic activities of mangrove extracts have been summarized in Table 2. Extracts with IC_{50} value < 100 µg/mL were considered to be cytotoxic, while those with IC_{50} value > 100 µg/mL were considered to be low/non cytotoxic.

3.1 Selective cytotoxic effects of mangrove extracts

Among the 116 extracts tested in MCF-7 and Hep G2 cells, chloroform extract of stem bark of *Scyphiphora hydrophyllacea*, *Bruguiera gymnorrhiza* (chloroform, ethyl acetate and methanol extracts of leaves), hexane and ethyl acetate extracts of leaves of *Aegiceras corniculatum*, methanol extracts

of leaves and stem bark of *N. fruticans* and *Rhizophora mucronata,* methanol extracts of stem bark of *Sonneratia alba* and *Rhizophora apiculata* showed selective cytotoxicity to HepG2 cells. However, the hexane extract of leaves of *B. gymnorrhiza*, chloroform extracts of leaves of *N. fruticans*, ethyl acetate extract of stem bark of *Lumnitzera littorea*, chloroform extract of leaves of *R. apiculata* and chloroform extract of leaves of *Pemphis acidula* showed selective cytotoxic effects against MCF-7 breast cancer cells.

3.2 Non-selective cytotoxic effects of mangrove extracts

Hexane extracts of leaves and stem bark of *S. hydrophyllacea*, methanol extract of leaves of *P. paludosa* and ethyl acetate extract of stem bark of *A. officinalis* showed non-selective cytotoxic activity against both cancer cell lines tested.

3.3 Low or no cytotoxic effects of mangrove extracts

Out of the 116 mangrove extracts tested, 84 extracts showed no significant cytotoxic effects (IC_{50}>100 µg/mL) against MCF 7 and HepG2 cancer cells (Table 2).

Table 2. Cytotoxic effects of leaves and stem bark of mangrove plants and their voucher specimen numbers: IC_{50} values of plant extracts on MCF 7 and HepG2 cells as determined by SRB assay at 24 and 48 h post incubation periods.

4. Discussion

Mangrove forests are considered to be the most productive ecosystems in the world [63]. However, mangroves grow under conditions such as high salinity, strong winds, extreme tides, high temperatures and extreme muddy soils etc. Thus, mangrove plants possess physiological, biological, ecological and morphological adaptations to extreme conditions [64]. Even though mangrove

ecosystems have been studied broadly, there is a critical need to understand them better and care must be taken to prevent degradation and destruction of mangrove eco systems.

Results of the present study with the leaf and stem bark extracts of fifteen mangrove species grown in Sri Lanka indicates that some of them have cytotoxic properties in breast (MCF -7) and hepatocellular carcinoma (HepG2) cells. Some mangrove plant extracts showed selective cytotoxic effects against breast and hepatocellular carcinoma cells, whereas some extracts showed non-selective cytotoxicity against both cancer cell lines or were not cytotoxic (IC_{50} >100 µg/mL) against any of the cell lines tested. Among the extracts tested, the methanolic extract of *P. paludosa* leaves showed the highest cytotoxicity in the two cancer cell lines tested. We have previously shown cytotoxic activity of different leaf extracts (hexane, chloroform, ethyl acetate and methanol) of *P. paludosa* in several cancer cell lines and normal cell lines [65]. Ethyl acetate extract of *A. officinalis* stem bark showed second

highest cytotoxic properties in the two cancer cell lines tested. Previous studies by other researchers have shown that, *A. officinailis* leaf extracts have cytotoxic effects to Ehrlich ascites carcinoma (EAC) and human promyelocytic leukemia cell lines (HL 60) [66]. Hexane extract of *S. hydrophyllacea* leaves showed third highest cytotoxic properties in the two cancer cell lines tested and several cytotoxic compounds have been reported to be isolated from the mangrove plant *S. hydrophyllacea* [67]. *S. hydrophyllacea* (hexane and chloroform extracts of stem bark), *B. gymnorrhiza* (chloroform, ethyl acetate and methanol extracts of leaves), *Aegiceras coniculatum* (hexane and ethyl acetate extracts of leaves), *N. fruticans* (methanol extract of leaves and stem bark), *S. alaba* (methanol extract of stem bark), *A. officinalis* (methanol extract of bark), *R. apiculata* (methanol extract of stem bark) and *R. mucronata* (methanol extracts of leaves and stem bark) showed selective cytotoxic properties to HepG2 cells. Moreover, *B. gymnorrhiza* (hexane extract of leaves), *N. fruticans* (chloroform extract of leaves), *L. littorea* (ethyl acetate extract of stem bark), *R. apiculata* (chloroform extract of leaves) and *P. acidula* (chloroform extract of leaves) showed selective cytotoxic effects against MCF-7 breast cancer cells (IC_{50}< 100 µg/mL). Among these plants, *A. corniculatum*, which was cytotoxic to HepG2 cells, has been used as a medicinal plant in Bangladesh for asthma, diabetes and rheumatism. Extracts of this plant have reported to be cytotoxic to human gastric adenocarcinoma cells (AGS), colorectal adenocarcinoma cells (HT-29) and breast carcinoma cells (MDA-MB-435S) [68]. *Avicennia officinalis* and *B. gymnorrhiza* which was cytotoxic to HepG2 cells in the present study have been used in traditional medicine to treat for leprosy, hepatitis, as a diuretic and for eye disease respectively. Extracts of these plants have also shown cytotoxic properties in cancer cells [69]. None of the extracts obtained from *Lumnitzera racemosa, Heritiera littoralis, Excoecaria indica* and *Sonneratia caseolaris* showed significant cytotoxic properties (IC_{50} >100 µg/mL) in the two cancer cell lines tested.

This is the first study on screening of cytotoxic properties of leaf and bark of 15 listed mangrove plants grown in Sri Lanka against human breast and hepatocellular cancer cell lines. This study supports the reported cytotoxic activities of *S. hydrophyllacea*, *A. corniculatum*, *A. officinalis* and *B. gymnorrhiza.* Cytotoxic properties of *B. gymnorrhiza*, *P. paludosa*, *N.fruticans*, *S. alba*, *L. littorea*, *R. apiculata*, *R. mucronata* and *P. acidula* have not been reported previously. This study offers baseline data to focus on further studies into the isolation and characterization of novel secondary metabolites and to

determine anti-cancer mechanism of such metabolites from mangrove plants grown in Sri Lanka. Mangrove plants that were found to be cytotoxic in the present study will be very useful as a source of new anti- cancer drug leads for drug discovery to fight against cancer.

5. CONCLUSION

Screening of leaves and stem barks of 15 selected mangrove plants growing in Sri Lanka, for cytotoxic activity in MCF -7 breast cancer cells and HepG2 hepatocellular carcinoma cells have demonstrated. Some mangrove plant extracts can exert selective cytotoxic properties to MCF -7 and

HepG2 cells, whereas a few plant extracts showed non-selective cytotoxic properties, while a few others demonstrated no cytotoxic properties. The overall results indicate that some mangrove species found in Sri Lanka have the potential to be developed to isolate novel drugs that can be used in cancer therapy.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

REFERENCES

1. Lugo AE, Snedaker SC. The ecology of mangroves. Annu Rev Ecol Evol Syst. 1974;1:39-64.

2. Duke NC, Meynecke JO, Dittmann S, Ellison AM, Anger K, Berger U, Cannicci S, Diele K, Ewel KC, Field CD, Koedam N. A world without mangroves?. Science. 2007;317(5834):41-2.

3. Sri Lanka's Mangroves, Coast conservation Department in Sri Lanka, Sri Lanka.

4. Giri C, Zhu Z, Tieszen LL, Singh A, Gillette S, Kelmelis JA. Mangrove forest distributions and dynamics (1975–2005) of the tsunami-affected region of Asia J Biogeogr. 2008 ;35(3):519-28.

5. Lugo AE, Snedaker SC. The ecology of mangroves. Annu Rev Ecol Evol Syst. 1974;1:39-64.

6. Scholander PF, Hammel HT, Hemmingsen E, Garey W. Salt balance in mangroves. Plant Physiol. 1962;37(6):722.

7. Zhang FQ, Wang YS, Lou ZP, Dong JD. Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). Chemosphere. 2007;67(1):44-50.

8. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetl Ecol Manag. 2002;10(6):421-52.

9. Alongi DM. Present state and future of the world's mangrove forests. Environmental conservation. 2002;29(03):331-49.

10. Ravikumar S, Inbaneson SJ, Suganthi P, Venkatesan M, Ramu A. Mangrove plants as a source of lead compounds for the development of new antiplasmodial drugs from South East coast of India. Parasitol Res. 2011;108(6):1405-10.

11. Balasooriya SJ, Sotheeswaran S, Balasubramanium S. Economically useful plants of Sri Lanka. Part IV. Screening of Sri Lanka plants for tannins. J Nat Sci Counc Sri Lanka. 1982; 10: 213–219.

12. Basak UC, Das AB, Das P. Chlorophyll, carotenoids, proteins and secondary metabolites in leaves of 14 species of mangroves. Bull Mar Sci. 1996; 58: 654–659.

13. Gomez ED, De La Cruz AA, Joshi BS, Chittawong V, Miles DH. Toxicants from mangrove plants, V. Isolation of piscicide 2-hydroxy-5-methoxy-3-undecyl-1,4- benzoquinone (5–0-methylembelin) from *Aegiceras corniculatum*. J Nat Prod. 1989; 52: 649–651.

14. Hensens OD, Lewis KG. Extractives of the bark of *Aegiciras corniculatum.* Aust J Chem. 1966;19: 169–174.

255
256 15. Popp M. Chemical composition of Australian mangroves. II. Low molecular weight carbohydrates.
257 Zeitschr Pflanzen. 1984; 113: 411–421.
258
259 16. Popp M, Larher F, Weigel P. Chemical composition of Australian mangroves. III. Free amino
260 acids, total methylated onium compounds and total nitrogen. Zeitschr Pflanzen. 1984; 114: 15–25.
261
262 17. Venkateswara Rao K, Bose PK. Chemistry of *Aegiceras majus Gaertn*-III: Structure of
263 aegiceradol. Tetrahedron. 1962; 18: 461–464.
264
265 18. Balasooriya SJ, Sotheeswaran S, Balasubramanium S. Economically useful plants of Sri Lanka.
266 Part IV. Screening of Sri Lanka plants for tannins. J Nat Sci Counc
267 Sri Lanka. 1982;10: 213–219.
268
269 19. Basak UC, Das AB, Das P. Chlorophyll, carotenoids, proteins and secondary metabolites in
270 leaves of 14 species of mangroves. Bull Mar Sci. 1996; 58: 654–659.
271
272 20. Fauvel MT, Bousquet Melou A, Moulis C, Gleye J, Jensen SR. Iridoid glycosides from *Avicennia*
273 *germinans.* Phytochemistry.1995; 38: 893–894.
274
275 21. Ghosh A, Misra S, Dutta AK, Choudhury A. Pentacyclic triterpenoids and sterols from seven
276 species of mangrove. Phytochemistry. 1985; 24: 1725–1727.
277
278 22. Madhu K , Madhu R. Biotoxicity of mangroves on fingerlings of *Liza macrolepis* (Smith). J
279 Andaman Sci Assoc Port Blair . 1997;13: 59–65.
280
281 23. Saxena H. A survey of the plants of Orissa (India) for tannins, saponins, flavonoids and alkaloids.
282 Lloydia. 1975; 38: 346–351.
283
284 24. Sharma M, Garg HS. Iridoid glycosides from *Avicennia officinalis*. Indian J Chem. 1996;35: 459–
285 462.
286

13
25. Achmadi S, Syahbirin G, Choong ET, Hemingway RW. Catechin-3-O rhamnoside 287 chain extender
288 units in polymeric procyanidins from mangrove bark. Phytochemistry. 1994; 35: 217–219.
289
290 26. Balasooriya SJ, Sotheeswaran S, Balasubramanium S. Economically useful plants of Sri Lanka.
291 Part IV. Screening of Sri Lanka plants for tannins. J Nat Sci Counc
292 Sri Lanka. 1982; 10: 213–219.
293
294 27. Ganguly SN, Sircar SM. Gibberellins from mangrove plants. Phytochemistry.1974; 13: 1911–
295 1913.
296
297 28. Ravi AV, Kathiresan K. Seasonal variation in gallotannin from mangroves. Indian J Mar Sci. 1990;
298 25: 142–144.
299
300 29. Iqbal AM, Hasan S, Uddin MJ, Rahman SA, Masud MM. Antinociceptive and Antioxidant Activities
301 of the Ethanolic Extract of *Excoecaria indica*. Dhaka Universirty. J Pharm Sci. 2007; 6 (1): 51-53.
302
303 30. Bagchi S, Matilal A, Shaw AK, Mukherjee BB. Lipids and waxes in some mangrove plants of
304 Sunderban, India. Indian J Mar Sci. 1988; 17: 150–152.
305

306 31. Balasooriya SJ, Sotheeswaran S, Balasubramanium S. Economically useful plants of Sri Lanka.
307 Part IV. Screening of Sri Lanka plants for tannins. J Nat Sci Counc Sri Lanka. 1982; 10: 213–219.
308
309 32. Popp M. Chemical composition of Australian mangroves. II. Low molecular weight carbohydrates.
310 Zeitschr Pflanzen. 1984; 113: 411–421.
311
312 33. Popp M, Larher F, Weigel P. Chemical composition of Australian mangroves. III. Free amino
313 acids, total methylated onium compounds and total nitrogen. Zeitschr Pflanzen. 1984; 114: 15–25.
314
315 34. Saad S, Taher M, Susanti D, Qaralleh H, Rahim NA. Antimicrobial activity of mangrove plant
316 (*Lumnitzera littorea*). Asian Pac J Trop Med. 2011 ;4(7):523-5.
317
318 35. Balasooriya SJ, Sotheeswaran S, Balasubramanium S. Economically useful plants of Sri Lanka.
319 Part IV. Screening of Sri Lanka plants for tannins. J Nat Sci Counc Sri Lanka. 1982; 10: 213–219.
320
321 36. Lin TC, Hsu FL, Cheng JT. Antihypertensive activity of corilagin chebulinic acid and tannins from
322 *Lumnitzera racemosa*. J Nat Prod. 1993; 56: 629–632.
323
324 37. Premnathan M, Chandra K, Bajpai SK, Kathiresan K. A survey of some Indian marine plants for
325 antiviral activity. Botanica Marina. 1992; 35: 321–324.
326
327 38. Rollet B. Bibliography on mangrove research. UNESCO Paris. Pub. Information Retrieval Ltd.,
328 London;1981.
329
330 39. Paeivoeke A, Adams MR, Twiddy DR. Nipa palm vinegar in Papua New Guinea. Proc Biochem.
331 1984; 19: 84–87.
332
333 40. Rollet B. Bibliography on mangrove research. UNESCO Paris. Pub. Information Retrieval Ltd.,
334 London; 1981.
335
336 41. Bourdy G, Francois C, Andary C, Boucard M. Maternity and medicinal plants in Vanuatu. II.
337 Pharmacological screening of five selected species. J Ethanopharm. 1996;
338 52: 139–143.
339
340 42. Rollet B. Bibliography on mangrove research. UNESCO Paris. Pub. Information Retrieval Ltd.,
341 London; 1981.
342
343 43. Lima AA, Parial R, Das M, Kumar AD. Phytochemical and Pharmacological studies of ethanolic
344 extract from the leaf of Mangrove plant *Phoenix paludosa Roxb*. Malayasian J Pharmaceut Sci.
345 2010; 8(2): 59-69.

346
347 44. Samarakoon SR, Shanmuganathan C, Ediriweera MK, Tennekoon KH, Piyathilaka P, Thabrew I,
348 de Silva ED. In vitro Cytotoxic and Antioxidant Activity of Leaf Extracts of Mangrove Plant, *Phoenix*
349 *paludosa Roxb*. Trop J Pharm Res. 2016 ;15(1):127-32.
350
351 45. Balasooriya SJ, Sotheeswaran S, Balasubramanium S. Economically useful plants of Sri Lanka.
352 Part IV. Screening of Sri Lanka plants for tannins. J Nat Sci Counc Sri Lanka. 1982; 10: 213–219.
353
354 46. Kato A. Brugine from *Bruguiera cylindrica.* Phytochemistry. 1975; 14: 1458.
355
356 47. Thangam TS, Kathiresan K. Toxic effect of mangrove plant extracts on mosquito larvae

357 *Anopheles-Stephensi L*. Current Science. 1988; 57: 914–915.
358
359 48. Thangam TS Kathiresan K. Mosquito larvicidal activity of mangrove plant extracts and synergistic
360 activity of *Rhizophora apiculata* with pyrethrum against *Culex quinquefasciatus*. Int J Pharma. 1997;
361 35: 1–3.
362
363 49. Balasooriya SJ, Sotheeswaran S, Balasubramanium S. Economically useful plants of Sri Lanka.
364 Part IV. Screening of Sri Lanka plants for tannins. J Nat Sci Counc Sri Lanka. 1982; 10: 213–219.
365
366 50. Basak UC, Das AB, Das P. Chlorophyll, carotenoids, proteins and secondary metabolites in
367 leaves of 14 species of mangroves. Bull Mar Sci. 1996; 58: 654–659.
368
369 51. Seshadri TR, Trikha RK. Procyanidins of *Ceriops roxburghiana* and *Rhizophora conjugata*. Indian
370 J Chem. 1971; 9: 928–930.
371
372 52. Shinoda Y, Ogisu M, Iwata S, Tajima T. Chemical composition of mangroves. 11. Gifu Daigaku
373 Nogakubu Kenkyu Hokoku. 1985; 50: 155–165.
374
375 53. Samarakoon SR, Fernando N, Ediriweera MK, Adhikari A, Wijayabandara L, de Silva ED,
376 Tennekoon KH. Isolation of Hopenone-I from the Leaves of Mangrove Plant *Scyphiphora*
377 *hydrophyllacea* and Its Cytotoxic Properties. British Journal of Pharmaceutical Research. 2016;10:1-6.
378
379 54. Zeng YB, Mei WL, Zhuang L, Hong K, Dai HF. Cytotoxic components from mangrove plant
380 *Scyphiphora hydrophyllacea*. J Trop Subtrop Bot. 2007;15:249-52.
381
382 55. Balasooriya SJ, Sotheeswaran S, Balasubramanium S. Economically useful plants of Sri Lanka.
383 Part IV. Screening of Sri Lanka plants for tannins. J Nat Sci Counc Sri Lanka. 1982; 10: 213–219.
384
385 56. Popp M. Chemical composition of Australian mangroves. II. Low molecular weight carbohydrates.
386 Zeitschr Pflanzen. 1984;113: 411–421.
387
388 57. Rollet B. Bibliography on mangrove research. UNESCO Paris. Pub. Information Retrieval Ltd.,
389 London;1981.
390
391 58. Devi P, Solimabi W, D'Souza L, Kamat SY. Toxic effects of coastal and marine plant extracts on
392 mosquito larvae. Botanica Marina. 1997; 40: 533–535.
393
394 59. Hogg RW, Gillan FT. Fatty acids, sterols and hydrocarbons in the leaves from eleven species of
395 mangrove. Phytochemistry. 1984; 23: 93–97.
396
397 60. Rollet B. Bibliography on mangrove research. UNESCO Paris. Pub. Information Retrieval Ltd.,
398 London;1981.
399
400 61. Samarakoon SR, Thabrew I, Galhena PB, De Silva D, Tennekoon KH. A comparison of the
401 cytotoxic potential of standardized aqueous and ethanolic extracts of a polyherbal mixture comprised
402 of *Nigella sativa* (seeds), *Hemidesmus indicus* (roots) and *Smilax glabra* (rhizome). Pharmacog Res.
403 2010; 2(6): 335.

62. Ediriweera MK, Tennekoon KH, Samarakoon SR, Thabrew I, Dilip De Silva 404 E. A study of the
405 potential anticancer activity of *Mangifera zeylanica* bark: Evaluation of cytotoxic and apoptotic effects
406 of the hexane extract and bioassay-guided fractionation to identify phytochemical constituents. Oncol
407 Lett. 2016;11(2):1335-44.

408
409 63. Bandaranayake WM. Traditional and medicinal uses of mangroves. Mangroves and salt marshes.
410 1998; 2(3):133-48.
411
412 64. Ball MC. Salinity tolerance in the mangroves *Aegiceras corniculatum* and *Avicennia marina*. I.
413 Water use in relation to growth, carbon partitioning, and salt balance. Funct Plant Biol. 1988
414 ;15(3):447-64.
415
416 65. Samarakoon SR, Shanmuganathan C, Ediriweera MK, Tennekoon KH, Piyathilaka P, Thabrew I,
417 de Silva ED. In vitro Cytotoxic and Antioxidant Activity of Leaf Extracts of Mangrove Plant, *Phoenix*
418 *paludosa Roxb*. Tropical Journal of Pharmaceutical Research. 2016 ;15(1):127-32.
419
420 66. Das G, Gouda S, Mohanta YK, Patra JK. Mangrove plants: A potential source for anticancer
421 drugs. Indian J Mar SCI. 2015 ; 44(5).
422
423 67. Zeng YB, Mei WL, Zhuang L, Hong K, Dai HF. Cytotoxic components from mangrove plant
424 *Scyphiphora hydrophyllacea*. J Trop Subtrop Bot. 2007;15:249-52.
425
426 68. Uddin SJ, Grice ID, Tiralongo E. Cytotoxic effects of Bangladeshi medicinal plant extracts. Evid
427 Based Complement Altern Med. 2009; 2011:1-7.
428
429 69. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of mangrove
430 plants. Wetl Ecol Manag. 2002;10(6):421-52.