Evaluation of Cytotoxic Potential of *Digera muricata* Leaf Extract on Lung Cancer Cell Line

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Authors’ contributions

This work was carried out in collaboration among all authors. *All authors read and approved the final manuscript.*

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ABSTRACT

Lung cancer is the second most frequent cancer, accounting for one out of every five male cancers and one out of every nine female cancers. Treatment for lung cancer is determined by the disease's cell type, the extent to which it has spread, and the patient's overall health. It is common knowledge that tumours impart resistance to chemotherapeutic medicines or radiation in part owing to apoptotic pathway dysfunction in cancer cells. *Digera muricata (D.muricata)* has been used as medicinal remedies for various ailments due to its antioxidant, anti-inflammatory, anti-bacterial, anti-tumor activity. The objective of the study was to examine the cytotoxic activity of ethanolic leaf extract of *D.muricata* on lung cancer cell lines. The cytotoxic potency of *D.muricata* leaf extract was carried out by MTT assay against the lung cancer cell line (A549). Different concentrations of *D.muricata* ethanolic leaf extract (25-150µg/ml) were treated for 24h. Furthermore, the morphological changes were analysed using phase contrast microscopy. Pro-apoptotic and nuclear morphological changes in *D. muricata* ethanolic leaf extract treated cells were examined using DAPI staining. The ethanolic leaf extract of *D.muricata* showed the dose dependent cytotoxic potency against the A549 cell line which confirmed with greater morphological changes upon 24 hrs treatment. The MTT assay clearly showed that the *D.muricata* treatment has significantly reduced the cell viability when the concentration was increased for 24hrs. We observed IC-50 dose

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at 50 μg/ml concentration. DAPI staining clearly showed condensed chromatin and fragmented nuclei in treated lung cancer cells. All these results clearly showed that ethanolic extract of D. muricata treatment significantly inhibited the cell proliferation and induced apoptosis in lung cancer cells.

Keywords: Digera muricata; ethanolic extract; lung cancer; cytotoxicity; apoptosis.

1. INTRODUCTION

With the increasing demand for natural alternatives to medical care, more plants are being studied worldwide for various medicinal benefits [1]. It is thus important that plants with therapeutic benefits are documented. Health care professionals and organizations are developing new methods and medicines abiding by the legacy of traditional medicine to replace the chemical treatment otherwise provided [2,3]. The adverse effects of long term drugs is the fulcrum sliding people to searching for alternatives such as natural or traditional medicine [4,5]. D. muricata is one such plant with various medicinal properties. D. muricata is also known as latmahuria. It is a wild and edible plant belonging to the Amaranthaceae family. Different parts of the D. muricata plant are known to serve different biological functions [6]. Urinary discharges are treated with the seeds and flowers [7]. It is also used as an astringent and laxative for the bowels. The roots of the herb are boiled and infused into water. This infusion is given to new mothers to improve lactation. The herb is most commonly used for renal disorders in folk medicine. There is also evidence to show that the free radicals cause injuries to the kidneys [8]. D.muricata is used to treat carbon tetrachloride induced toxicity [9].

Research on new medication to treat lung cancer is done using lung cancer cell lines. There is a current collection of more than 200 lung cancer cell lines. This collection was possible because of a systemic approach to initiate and characterise cell lines from small and non-small cell lung cancer. The lung cancer cell lines are widespread and are at ready disposal for use by any researcher. This allows multiple researches to be performed and helps catalyse a vast number of biomedical discoveries. There are major similarities between lung tumors and lung cancer cell lines [10]. The main purpose for research using lung cancer cell lines is due to the sharp increase in lung cancer cases over the century. Lung cancer is the most common cause of death due to cancer among women and men worldwide. The main causative or risk factors for lung cancer are smoking, asbestos, carbon inhalation and other occupational hazards like radon and environmental tobacco smoke [10]. In India, it is the second most common type of cancer which can be fatal to both males and females. Unlike other cancer diagnoses, lung cancer is usually detected in the later stages and is usually associated with distant metastasis among both males and females as of the statistics from 2020 [11].

Molecular and cellular pathogenesis have been studied in detail over the past few decades. It has been deduced that the changes of tumor suppressor genes and abnormal proto-oncogenes play a role in angiogenesis and development of lung cancer. Theories also suggest molecular abnormalities leading to respiratory lesions. It is predicted that new pathways and targets may be discovered in lieu of the complex genetic involvement for development of lung cancer [12]. This calls for newer clinical assessment methods and newer treatment options. New generation diagnosis for lung cancer involves the identification of early metabolic markers for early detection of lung cancer. In the near future, software tools like genomics, imageology, metabolomics need to be developed for analysing the mechanism of lung cancer [13]. Cancer is a lethal condition and needs holistic and systemic care to enter remission. Thus, preventive measures must be strictly followed and early cancer markers must be easily identified. Chemotherapy leads to various deleterious side effects and may also be a risk factor for future cancer. Due to such effects, popularity for natural cure and medication has recently increased [14]. Many plant concentrates have been discovered to have cytotoxic effects against various cancer cells [15]. Finding the most efficient plant extract for the specific cancer cell lines is of utmost importance [16-42]. Therefore, the present study is aimed to check for the cytotoxic effect of ethanolic leaf extract of D.muricata against lung cancer cell lines.
2. MATERIALS AND METHODS

2.1 Reagents

DMEM (Dulbecco’s Modified Eagle Medium), Phosphate Buffered Saline (PBS), Trypsin-EDTA, Fetal bovine serum (FBS), were purchased from Gibco, Canada. 4′,6-diamidino-2-phenylindole (DAPI), Dimethyl sulfoxide (DMSO), [3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT), were purchased from Sigma Chemical Pvt Ltd, USA. All other chemicals used were extra pure of molecular grade and were purchased from Sisco Research Laboratories (SRL), India.

2.2 Cell Line Maintenance

Lung cancer cell lines (A549) were obtained from the National Centre for Cell Sciences (NCCS), Pune. The cells were grown in T25 culture flasks containing DMEM supplemented with 10% FBS and 1% antibiotics. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂. Upon reaching confluency, the cells were trypsinized and passaged.

2.3 Preparation of the Herbal Extract

Leaf of D. muricata obtained from The Indian Medical Practitioners Co-operative Pharmacy and Stores (IMPCOPS) (Chennai, India) was used for the present study. About 50g of D. muricata powder was soaked in 500 mL of 95% ethanol and kept at room temperature for 3 days in a static condition. Then the solution was filtered with crude filter paper followed by whatmann paper. Fine filtrate was subjected to rota evaporation after that 3g of the material was obtained. The total ethanol extract was concentrated in a vacuum evaporate and immediately stored at 4°C.

2.4 Cell Viability (MTT) Assay

The cell viability of plant extract treated A549 cells was assessed by MTT assay. The assay is based on the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically active cells. A549 cells were plated in 48 well plates at a concentration of 2×10⁴ cells/well 24 hrs after plating, cells were washed twice with 500µl of serum-free medium and starved by incubating the cells in serum-free medium for 3 hours at 37°C. After starvation, cells were treated with different concentrations of D. muricata ethanolic leaf extract (25 - 150µg) for 24 hrs. At the end of treatment, the medium from control and treated cells were discarded and 200µl of MTT containing DMEM (0.5 mg/ml) was added to each well. The cells were then incubated for 4h at 37°C in the CO₂ incubator. The MTT containing medium was then discarded and the cells were washed with 1x PBS. The crystals were then dissolved by adding 200µl of solubilization solution and this was mixed properly by pipetting up and down. Then the formazan crystals formed were dissolved in dimethyl sulfoxide (200µl) and incubated in dark for an hour. Then the intensity of the color developed was assayed using a Micro ELISA plate reader at 570 nm. The number of viable cells was expressed as the percentage of control cells cultured in serum-free medium. Cell viability in the control cells without any treatment was represented as 100%. The cell viability is calculated using the formula: % cell viability = [A570 nm of treated cells/A570 nm of control cells]×100.

2.5 Morphology Study

Based on MTT assay we selected the optimal doses (IC-50: 50µg/ml) for further studies. Analysis of cell morphology changes by a phase contrast microscope. 3×10⁴ cells were seeded in 6 well plates and treated with D. muricata (50µg/ml) for 24h. At the end of the incubation period, the medium was removed and cells were washed once with a phosphate buffer saline (PBS pH 7.4). The plates were observed under a phase contrast microscope.

2.6 Determination of Nuclear Morphological Changes of Cells (DAPI Staining)

For the nuclear morphological analysis, after the treatment period, the monolayer of cells was washed with PBS and fixed with 3% paraformaldehyde for 10 min at room temperature. The fixed cells were permeabilized with 0.2% Triton X-100 in PBS for 10 min at room temperature and incubated with 0.5µg/ml of DAPI for 5 min. The apoptotic nuclei (intensely stained, fragmented nuclei, and condensed chromatin) were viewed under a fluorescent microscope.

2.7 Statistical Analysis

All data obtained were analyzed by Student's-t-test using MS-Excel, represented as mean ± SD.
for triplicates. The level of statistical significance was set at p<0.05.

3. RESULTS

3.1 Effect of *D. muricata* Leaf Extract on the Cell Viability of Lung Cancer Cell Line

MTT assay was used to assess the cytotoxic potential of the leaf extract of *D. muricata* on the lung cancer cell lines. Various concentrations of the extract (25-150 μg/ml) were tested against the lung cancer cell line for 2 hrs. The results of the assay suggested that there was a significant decrease in the viability of lung cancer cell lines (A549) when treated with *D. muricata* leaf extract when compared to the control for 24 hours. The viability of the cells gradually decreased with increasing concentration of the leaf extract. It was observed that 50% growth inhibition occurred at a concentration of 50 μg/ml. Thus, the IC-50 dose considered for further experiments was 50 μg/ml. (Fig. 1)

3.2 The Effect of *D. muricata* Leaf Extract on Cell Morphology

Phase contrast microscopy was used to analyse the changes in cell morphology of the lung cancer cell lines when treated with *D. muricata* leaf extract. The changes in cell morphology among the lung cancer cell lines treated with 50 μg/ml concentration of *D. muricata* leaf extract was compared with the control group for 24 hrs. The results suggest that the treated cells showed characteristic changes similar to that of apoptotic cells- shrinkage of cells with reduced cell density. Some cells also showed other changes much like that of apoptotic cells like cell rounding and loss of contact with adjacent cells. Some cells detached themselves from the base of the plates (Fig. 2).

3.3 Pro-apoptotic Effect of *D. muricata* Leaf Extract in Lung Cancer Cell Lines (DAPI Staining)

DAPI staining is used to analyse the induction of apoptosis among the lung cancer cell lines treated with *D. muricata* leaf extract (50 μg/ml) after 24 hrs. Fluorescence microscopy was used to check the pro-apoptotic effect post staining. The results suggested that the cancer cell lines treated with the leaf extract showed condensation of chromatin along with nuclear fragmentation. These changes are suggestive of characteristic apoptotic activity when compared to the control group which had round nuclei (Fig. 3).

![MTT Assay](image_url)

**Fig. 1.** The cytotoxic effects of *D. muricata* on A549 cells was determined by MTT assay. The Cells were treated with different concentrations (0, 25, 50, 75, 100, 125 and 150 μg) for 24hrs. The 50% of inhibition observed in concentration of 50 μg/ml, (p value: 0.0215) which has been taken as IC50 value and fixed for further experiments. Data are shown as means ± SD (n = 3). * compared with the control-blank group, p < 0.001
Fig. 2. Represents the morphological changes in lung cancer cell line upon without and with treatment of D.muricata at 50 μg/mL for 24hrs by phase contrast microscope at 20x magnification

Fig. 3. Induction of apoptosis in D. muricata leaf extract treated lung cancer cell line. The nuclei were stained with DAPI staining and observed under a fluorescence microscope. The treated cells clearly showed condensed chromatin and nuclear fragmentation

4. DISCUSSION

Based on the MTT assay, it can be inferred that there was a significant cytotoxic activity of the ethanolic extract of D.muricata against lung cancer cell lines. The cytotoxic potential of the ethanolic extract was found to be statistically significant in comparison with the control group. It was identified that the inhibitory concentration for this ethanolic extract of D.muricara is 50μg/ml. The cytotoxic potential of this plant extract may be owing to the presence of substances known as flavonoids, present in the ethanolic extract. The flavonoids are composed of mono or poly phenolic groups. These phenolic groups dispense the cytotoxic potential to flavonoids [43]. The results obtained from this study are supported by the results from previous studies. A study done by Mehwish et al compared the various bioactivities of different plant extracts. It was found that D. muricata had the most concentration of flavonoid content and thus best cytotoxic activity [44]. A similar study conducted in Bangladesh checked the various bioactivities of D.muricata and concluded that apart from its antidepressant, anti diarrhoea, analgesic activities, it also has good cytotoxic potential [45].

The cell morphology test compared the differences in cell morphology of the control and the cell morphology of the cell lines treated with the ethanolic extract of D.muricata. It was observed that the number of cells decreased after treatment and the cells exhibited cell shrinkage and cytoplasmic membrane blebbing.
Previous studies also analysed the changes in cellular morphology of the cancer cell line. Their studies also confirmed similar cellular changes with increased free floating cells [46,47]. The DAPI staining apoptosis test result suggests that there was condensation of chromatin and nuclear fragmentation which occurred owing to the process of apoptosis [48]. These changes were observed under fluorescent microscopy. These results obtained in our study are supported by previous similar studies done. Most anticancer effects are based on apoptotic activity of the extracts or the chemotherapeutic agents [49,50]. However, future in vivo studies need to be performed for better understanding and usage of the extract as a cytotoxic agent.

5. CONCLUSION

Overall, the present study results demonstrated that the plant extracts of D.muricata were cytotoxic and induced apoptosis to the lung cancer cells at 50µg/ml concentration for 24h incubation period. However more research is needed to understand the mechanisms of cytotoxicity of this plant extract.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lakshmi T, Ezhilarasan D, Nagaich U, Vijayaragavan R. Acacia catechu ethanolic seed extract triggers apoptosis of SCC-25 Cells. Pharmacogn Mag. 2017;13(Suppl 3):S405–11.
2. Lakshmi T, Ezhilarasan D, Vijayaragavan R, Bhullar SK, Rajendran R. Acacia catechu ethanolic bark extract induces apoptosis in human oral squamous carcinoma cells. J Adv Pharm Technol Res. 2017;8(4):143–9.
3. Ashwini S, Ezhilarasan D, Anitha R. Cytotoxic effect of Caralluma fimbriata against human colon cancer cells. Pharmacognosy Journal; 2017. Available: https://www.phcogj.com/article/252
4. Manimekalai P, Kaveena R, Naveena S, Nivetha S, Nivetha N. A review on pharmacognostical and phytochemical study of (Digera muricata L.). J Med Plants Stud. 2020;8(5):52–6.
5. Ezhilarasan D, Sokal E, Karthikeyan S. Plant derived antioxidants and antifibrotic drugs: past, present and future. J Coast Life; 2014; Available: https://www.researchgate.net/profile/Ezhilarasan_Devaraj/publication/302959897_Plant Derived Antioxidants_and Antifibrotic Drugs_Past_Present_and_Future/links/58c76528aca27232ac8d9026/Plant-derived-antioxidants-and-antifibrotic-drugs-Past-Present-and-Future.pdf
6. Parrotta JA. Healing plants of peninsular India. Parrotta JA, editor. Wallingford, England: CABI Publishing. 2001:944.
7. Kirtikar KR. Indian medicinal plants: By K.R. Kirtikar, B.D. Basu, and An I.C.S. In 4 volumes. Allahabad: Lalit Mohan Basu; 1935.
8. Khan MR, Rizvi W, Khan GN, Khan RA, Shaheen S. Carbon tetrachloride-induced nephrotoxicity in rats: protective role of Digera muricata. J Ethnopharmacol. 2009;122(1):91–9.
9. Khan MR, Ahmed D. Protective effects of Digera muricata (L.) Mart. on testis against oxidative stress of carbon tetrachloride in rat. Food Chem Toxicol. 2009;47(6):1393–9.
10. Gazdar AF, Girard L, Lockwood WW, Lam WL, Minna JD. Lung cancer cell lines as tools for biomedical discovery and research. J Natl Cancer Inst. 2010;102(17):1310–21.
11. Mathur P, Sathishkumar K, Chaturvedi M, Das P, Sudarshan KL, Santhappan S, et al. Cancer Statistics, 2020: Report From
National Cancer Registry Programme, India. JCO Glob Oncol. 2020;6:1063–75.

12. Zochbauer-Muller S, Gazdar AF, Minna JD. Molecular pathogenesis of lung cancer. Annu Rev Physiol. 2002;64:681–708.

13. Yu L, Li K, Zhang X. Next-generation metabolomics in lung cancer diagnosis, treatment and precision medicine: Mini review. Oncotarget. 2017;8(70):115774–86.

14. Ezhillarasan D. Herbal therapy for cancer. Understanding cancer therapies; 2018. Available: https://books.google.ca/books?hl=en&lr=&id=DnlQdwaAAQBAJ&oi=fnd&pg=PT142&ots=Vtb7T6j4r&sig=DUidLAFBuxy2olxALwJhlfJ67F4

15. Vignesh P, Rajeshkumar S, Lakshmi T. Cytotoxic effects of herbal formulation mediated silver nanoparticles. Drug Invention; 2019. Available: http://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authkey=09757619&AN=141706059&h=jvRFANiETMToOdqRQhOCeUuWtav5wiKv5iOL4Hkz7omGz5Ov6gggFceOLRmfsWGtrptbkuaw0ircsQjQ%3D%3D&cril=c

16. Sunar S, Rajeshkumar S, Roy A, Lakshmi T. Preparation of herbal formulation and it’s application on nanoparticles synthesis and antibacterial activity. International Journal of; 2019. Available: https://www.scilit.net/article/96a174f1aad99f99e115e6f70e04fc7

17. Abitha ST, Rajeshkumar S, Lakshmi T. Cytotoxic effects of silver nanoparticles synthesized using amla fruit seed. Drug Invention; 2019. Available: http://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authkey=09757619&AN=139165824&h=vtiz4ba1lO6RLaxvecoYuMzoQfPWA9ih%2B2Q2rIAsqTq%2B6GqhwMrFTH6TKkKdUL%2FePEDMZtvDwsfSpRD5r%2Bmw%3D%3D&cril=c

18. Rajeshkumar S, Kumar SV, Ramaiah A, Agarwal H, Lakshmi T, Roopan SM. Biosynthesis of zinc oxide nanoparticles using Mangifera indica leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. Enzyme Microb Technol. 2018;117:91–5.

19. Nandhini NT, Rajeshkumar S, Mythili S. The possible mechanism of eco-friendly synthesized nanoparticles on hazardous dyes degradation. Biocatal Agric Biotechnol. 2019;19:101138.

20. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of Enterococcus sp.—mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells. Environmental Science and ; 2020. Available: https://link.springer.com/article/10.1007/s11751-x

21. Komathi M, Prakash A, Rajkumar PV, Rajeshkumar S, Chandrasekaran R, Anbarasan PM. Green synthesis of silver nanoparticles using Gymnema sylvestre leaf extract and evaluation of its antibacterial activity. S Afr J Chem Eng. 2020;32:1–4.

22. Rajasekaran S, Damodharan D, Gopal K, Rajesh Kumar B, De Poure MV. Collective influence of 1-decanol addition, injection pressure and EGR on diesel engine characteristics fueled with diesel/LDPE oil blends. Fuel. 2020;277:118166.

23. Santhoshkumar J, Venkat Kumar S, Rajeshkumar S. Phyto-assisted synthesis, characterization and applications of gold nanoparticles—A review Biochemistry and Biophysics Reports. 2017;11:46–57.

24. Raj R K, D E, S R. β-Sitosterol-assisted silver nanoparticles activates Nrf2 and triggers mitochondrial apoptosis via oxidative stress in human hepatocellular cancer cell line. J Biomed Mater Res A. 2020;108(9):899–908.

25. Saravanan M, Arokiyaraj S, Lakshmi T, Pugazhendhi A. Synthesis of silver nanoparticles from Phenerochaete chrysosporium (MTCC-787) and their antibacterial activity against human pathogenic bacteria. Microb Pathog. 2018; 117:68–72.

26. Gheena S, Ezhillarasan D. Syringic acid triggers reactive oxygen species—mediated cytotoxicity in HepG2 cells. Hum Exp Toxicol. 2019;38(6):694–702.

27. Ezhillarasan D, Sokal E, Najimi M. Hepatic fibrosis: It is time to go with hepatic stellate cell-specific therapeutic targets. Hepatobiliary Pancreat Dis Int. 2018;17(3):192–7.

28. Ezhillarasan D. Oxidative stress is bane in chronic liver diseases: Clinical and experimental perspective. Arab J Gastroenterol. 2018;19(2):56–64.
29. Gomathi AC, Xavier Rajarathinam SR, Mohammed Sadiq A, Rajeshkumar S. Anticancer activity of silver nanoparticles synthesized using aqueous fruit shell extract of Tamarindus indica on MCF-7 human breast cancer cell line. J Drug Deliv Sci Technol. 2020;55:101376.

30. Dua K, Wadhwa R, Singhvi G, Rapalli V. The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress. Drug development; 2019. Available: https://onlinelibrary.wiley.com/doi/abs/10.1002/ddr.21571

31. Ramesh A, Varghese S, Jayakumar ND. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients—A case-control study. Journal of; 2018. Available: https://aap.onlinelibrary.wiley.com/doi/abs/10.1002/JPER.17-0445

32. Arumugam P, George R, Jayaseelan VP. Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma. Arch Oral Biol. 2021;122:105030.

33. Joseph B, Prasanth CS. Is photodynamic therapy a viable antiviral weapon against COVID-19 in dentistry? Oral Surg Oral Med Oral Pathol Oral Radiol. 2021;132(1):118–9.

34. Ezhilarasan D, Apoorva VS, Ashok Vardhan N. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med. 2019;48(2):115–21.

35. Duraisamy R, Krishnan CS, Ramasubramanian H, Sampathkumar J, Mariappan S, Navarasampatti Sivapakasam A. Compatibility of Nonoriginal Abutments With Implants: Evaluation of Micropat at the Implant–Abutment Interface, With Original and Nonoriginal Abutments. Implant Dent. 2019;28(3):289.

36. Gnanavel V, Roopan SM, Rajeshkumar S. Aquaculture: An overview of chemical ecology of seaweeds (food species) in natural products. Aquaculture; 2019. Available: https://www.sciencedirect.com/science/article/pii/S0044848618328072

37. Markov A, Thangavelu L, Aravindhan S, Zekiy AO, Jarahian M, Chartrand MS, et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. Stem Cell Res Ther. 2021;12(1):192.

38. veerasamy r, roy a, karunakaran r, rajak h. structure-activity relationship analysis of benzimidazoles as emerging anti-inflammatory agents: An overview. Pharmaceuticals. 2021;14(7). Available from: http://dx.doi.org/10.3390/ph14070663

39. An experimental analysis on the influence of fuel borne additives on the single cylinder diesel engine powered by Cymbopogon flexuosus biofuel. J Energy Inst. 2017;90(4):634–45.

40. Campeau PM, Kasperaviciute D, Lu JT, Burrage LC, Kim C, Hori M, et al. The genetic basis of DOORS syndrome: An exome-sequencing study. Lancet Neurol. 2014 ;13(1):44–58.

41. Sathish T, Karthick S. Wear behaviour analysis on aluminium alloy 7050 with reinforced SiC through taguchi approach. Vol. 9, Journal of Materials Research and Technology. 2020;3481–7. Available: http://dx.doi.org/10.1016/j.jmrt.2020.01.085

42. Krishnaswamy H, Muthukrishnan S, Thanikodi S, Árockiaraj G, Venkatraman V. Investigation of air conditioning temperature variation by modifying the structure of passenger car using computational fluid dynamics. Vol. 24, Thermal Science. 2020;495–8. Available: http://dx.doi.org/10.2298/tsci190409397k

43. Matsuo M, Sasaki N, Saga K, Kaneko T. Cytotoxicity of flavonoids toward cultured normal human cells. Biol Pharm Bull. 2005;28(2):253–9.

44. Mehwish S, Islam A, Ullah I, Wakeel A, Qasim M, Khan MA, et al. In vitro antileishmanial and antioxidant potential, cytotoxicity evaluation and phytochemical analysis of extracts from selected medicinally important plants. Biocatal Agric Biotechnol. 2019;19:101117.

45. Miah MM, Das P, Mridha SA, Kuddus MR, Rashid MA. Bioactivities of Digera muricata (L.) Mart. Available in Bangladesh. Dhaka University Journal of Pharmaceutical Sciences. 2018;16(2):251.

46. Elhussein A, Tynga I, El-Harith MA, Abrahame H. Photodynamic ability of silver nanoparticles in inducing cytotoxic effects in breast and lung cancer cell lines.
47. Manoto SL, Abrahamse H. Effect of a newly synthesized Zn sulfophthalocyanine derivative on cell morphology, viability, proliferation, and cytotoxicity in a human lung cancer cell line (A549). Lasers Med Sci. 2011;26(4):523–30.

48. Ezhilarasan D, Apoorva VS. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. Journal of Oral; 2019; Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/jop.12806

49. Usmani S. Anti-proliferative activity of crude extract and fractions obtained from digera muricata on HeLa cell lines of human cervix and A549 cell lines of human lung; 2018. [cited 2021 May 21]; Available from: http://dx.doi.org/

50. Rohit Singh T, Ezhilarasan D. Ethanolic extract of Lagerstroemia speciosa (L.) Pers., induces apoptosis and cell cycle arrest in HepG2 Cells. Nutr Cancer. 2020;72(1):146–56.

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