Journal of Pharmaceutical Research International

33(64A): 325-332, 2021; Article no.JPRI.74399
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Studies on Chemotherapeutic Potential of Hydroethanolic Leaf Extract of *Aegle marmelos* on Human Lung Cancer Cell Lines

N. Bharath Kumar [a], G. Sridevi [b≡*], R. Selvaraj [c≡] and S. Preetha [bφ]

[a] Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077, Tamil Nadu, India.
[b] Department of Physiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai-600077, Tamil Nadu, India.
[c] Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Velappanchavadi, Chennai-600077, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author NBK did the literature search, survey, experimental data collection, analysis and manuscript writing. Authors GS and RS did the study design, data verification and manuscript drafting. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i64A35706

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/74399

Original Research Article

Received 20 October 2021
Accepted 28 December 2021
Published 30 December 2021

ABSTRACT

Background: Lung cancer, one of the world's leading causes of cancer death. There is an improvement in mortality by screening of lung cancer by low dose computed tomography. Long time ago, lung cancer was a reportable disease which now became a commonest cause of death due to cancer. Discovery of medicine for various diseases is still an ongoing process. A lot of diseases are still remaining a threat without any treatment or medicine. Herbal derived medicines are preferred because of their low side effects on patients.
Objective: To investigate the role of *Aegle marmelos* against human lung cancer cell lines (A549 cell line).
Materials and Methods: A549 cell lines were procured from NCCS (National center for cell sciences) Pune, India. It was cultured and viability of the cells before and after adding the extract

≡Associate Professor;
φAssistant Professor;
**Corresponding author: E-mail: sridevig.sdc@saveetha.com;*

was analysed using the MTT assay. mRNA amplification was done using real time PCR.Statistical analysis was done using ANOVA and dunken's multiple test. Corresponding graphs are also plotted.

Results: The viability of the cells decreased from 100% to 50%. The mRNA expression of wnt and β-catenin decreased after the addition of the extract.

Conclusion: The study concluded that *Aegle marmelos* possesses a good, novel, innovative, anti-cancer activity against the lung cancer cell lines A549.

Keywords: Lung cancer; drug; innovative; Aegle marmelos; cell lines; cell viability.

1. INTRODUCTION

Cancer is the second leading cause for death following cardiovascular diseases in India. Herbal plants are usually used by many traditional healers to treat various symptoms and signs of diseases including fever, cold, headache, diabetes, diarrhea, and now even cancer. It is very clear that now plants have a very useful clinical effect and have an antitumor activity [1,2]. WHO has given a statistic of 70-95% of the developing world's population relying on various traditional plants based medicine systems and healthcare. Cancer cells have the ability to escape apoptosis in many ways [3,4]. The main function of anticancer drugs is to prevent it from escaping apoptosis and stop it from proliferating more. These drugs are set to induce cell apoptosis in tumour cells by having cytotoxic effect and hence remains a vital treatment for cancer [5,6,7]. A few drugs that perform the above action are 5-fluorouracil, cisplatin, etoposide, VM26, etc. [8,1]. Thus the identification of potential chemotherapeutic agents using mechanism based studies holds great promise for elucidating mechanisms and devising more specific and effective treatments for cancer related diseases [9,10,1,2,11].

One such approach of discovery is the ethnomedical data approach, where plant selection is based on prior information on the folk medicinal use of plants. It is generally known that this data substantially increases the chance of finding active plants related to random approaches [12]. The plant selected for present study – *Aegle marmelos*. There is no recorded data for cytotoxicity against cancer cell lines and normal cell lines for these plants [13,14]. Previous studies have concluded that the solvent extracts of the above plant-*Aegle marmelos* were screened for in-vitro antioxidant, antiproliferative, anti-inflammatory and anticancer activities [15,16]. This research focuses on the chemotherapeutic potential of hydro ethanolic extract of *Aegle marmelos* on human lung cancer cell lines.

2. MATERIALS AND METHODS

Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Pvt Ltd, USA. Trypsin-EDTA, fetal bovine serum (FBS), antibiotics-antimycotics, RPMI 1640 medium and phosphate buffered saline (PBS) were purchased from Gibco, Canada. (5,5,6,6-tetrachloro-1,1,3,3-tetraethyl benzimidazole carbocyanine iodide) and Real Time PCR kit was purchasedTAKARA (Meadowvale Blvd, Mississauga, ON L5N 5S2, Canada).

Study design: *In vitro* study.

Duration: 3 months.

No ethical consideration involved as it is an *in vitro* study in the laboratory.

Inclusion criteria: The study involved Human lung cancer cell lines for *in vitro* based studies and cell viability assays to evaluate anticancer activity of *Aegle marmelos* through Wnt-mRNA expression of A549 and β- Catenin-mRNA expression of A549.

Exclusion criteria: The study excluded the involvement of aqueous and other solvent extracts for anticancer activity.

2.1 Cell Lines and Cell Culture

The Human Lung cancer cell line (A549) was purchased from the National Centre for Cell Sciences (NCCS), Pune, India. Cells were cultured in RPMI 1640 medium (Thermo Fisher Scientific, CA, USA) containing 10% fetal bovine serum (Thermo Fisher Scientific, CA, USA), 100 U/ml penicillin and 100 µg/ml streptomycin (Thermo Fisher Scientific, CA, USA) at 37°C with 5% CO_2.

Table 1. Primer sequence

S. No	Gene	Primer sequence
2	Human	Forward: 5′-CTTACACCCACCATCCCA CT-3′
	β-catenin	Reverse: 5′-CCTCCACAAATTGCTGCTGT-3′
3	Human	Forward:5′-GCCGTGTCATGCTCAGAA-3′
	Wnt	Reverse: 5′-GTG GAC TAC CCC TGC TGA TG-3′
4	Human	Forward:5'-CTACAATGAGCTGCGTGTGG -3'
	β-actin	Reverse: 5'TAGCTCTTCTCCAGGGAGGA-3'

2.2 Cell Viability by MTT Assay

Cell viability was assayed employing a modified colorimetric technique that supported the power of live cells to convert MTT, a tetrazolium compound into purple formazan crystals by mitochondrial reductases (Mosmann, 1983). Briefly, the cells (1 ×104/well) were exposed to different concentrations of *Aegle marmelos* extract (100-500µg/ml) with A549 cells for48 h. At the end of the treatment, 100 µl of 0.5 mg/ml MTT solution was added to each well and incubated at 37 ◦C for an hour. The formed formazan crystals were dissolved in dimethyl sulfoxide (100 µl) and incubated in dark for an hour. Then the intensity of the colour developed was assayed employing a Micro ELISA plate reader at 570 nm. The number of viable cells was expressed because the percentage of control cells cultured in serum-free medium. Cell viability on top of things medium with none treatment was represented as 100%. The cell viability is calculated using the formula: red blood cell viability = [A570 nm of treated cells/A570 nm of control cells] × 100.

2.3 Gene Expression Analysis by Real Time-PCR

Samples from each group were submerged in 2 ml Trizol (Invitrogen, Carlsbad, CA, USA) for RNA extraction and stored at −80°C until further processed. cDNA synthesis was performed on 2 µg RNA in a 10 µl sample volume using Superscript II reverse transcriptase (Invitrogen) as recommended by the manufacturer. Real-time PCR array analysis was performed in a total volume of 20 µl including 1 µl cDNA, 10 µl qPCR Master Mix 2x (Takara, USA) and 9 µl ddH2O. Reactions were run on an CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA) using universal thermal cycling parameters (95°C for 5 min, 40 cycles of 15 sec at 95°C, 15 sec at 60°C and 20 sec at 72°C; followed by a melting curve: 5 sec at 95°C, 60 sec at 60°C and continued melting). For internal control purposes, melting curves were acquired for all samples. The specificity of the amplification product is decided by melting curve analysis for every primer pair. The data were analyzed by comparative CT method and the fold change is calculated by 2−ΔΔCT method described bySchmittgen and Livak (2008) using CFX Manager Version 2.1 (Bio Rad, USA).

2.4 Statistical Analysis

The obtained data were analyzed statistically by one-way analysis of variance (ANOVA) and Duncan's multiple range test with a computer-based software (Graph Pad Prism version 5) to analyze the significance of individual variations among the control and experimental groups. The significance was considered at $p<0.05$ level in Duncan's test.

3. RESULTS

The viability of cancer cells which were 100% viable, after addition of *Aegle marmelos* extract decrease in viability based on the dosage was observed. It is observed to decreased to 50% viability when the concentration of the extract increased upto to 500 microgram (Fig. 1). It was also observed that there was a fold change over control of the Wnt-mRNA expression of A549, which decreases significantly on the addition of *Aegle marmelos* extract (Fig. 2). It was observed that there was a fold change over control of the β- Catenin-mRNA expression oh A549, which decreases significantly on the addition of *Aegle marmelos* extract (Fig. 3).

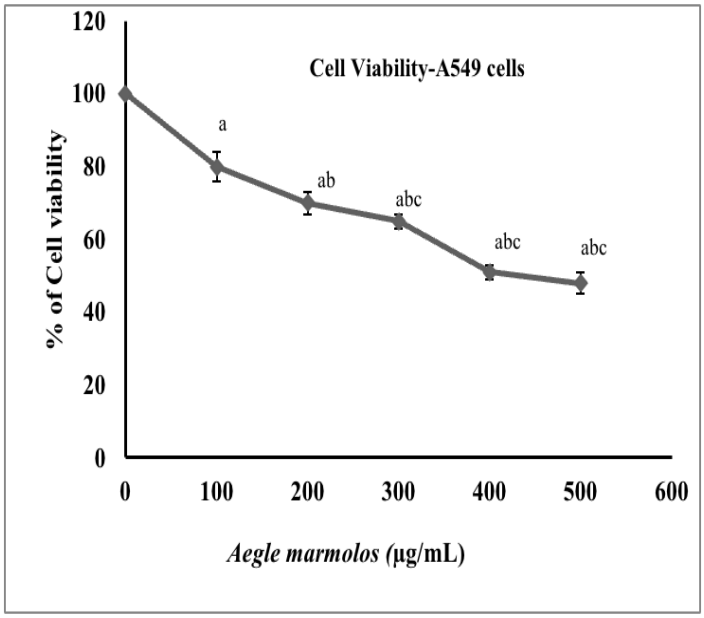

Fig. 1. Represents the effect of*Aegle marmelos* leaf extract on cell viability in A549 cells. Each bar represents a mean ± SEM of 6 observations. Significance at p< 0.05, a-compared with untreated control cells, b-compared with 1nM treated A549 cells, c-compared with 300µg treated cells. X-axis represents the concentration of extract and Y-axis represents the percentage of cell viability. There is a significant decrease in cell viability

3.1 Gene Expression Analysis

Wnt mRNA expression (Fold change over control).

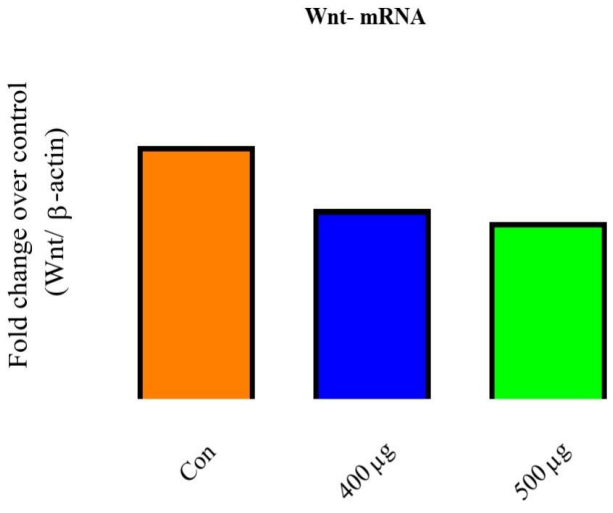

Fig. 2. Shows the effect of *Aegle marmelos* leaf extract on Wnt mRNA expression in A549 cells. Each bar represents a mean ± SEM of 6 observations. Significance at p< 0.05, a-compared with untreated control cells. X-axis represents the concentration of extract added and Y-axis represents the fold change over control of the mRNA expression of A549 cell lines.
β- Catenin- mRNA expression (Fold change over control)

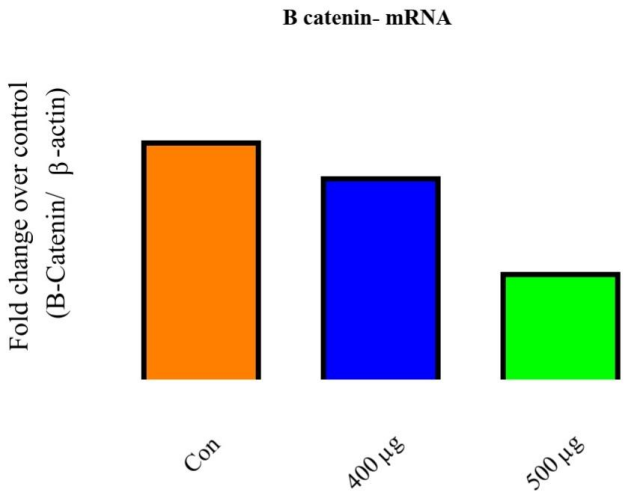

Fig. 3. Represents the effect of *Aegle marmelos* leaf extract on β- Catenin mRNA expression in A549 cells. Each bar represents a mean ± SEM of 6 observations. Significance at p< 0.05, a- compared with untreated control cells, b-compared with 400µg treated cells. X-axis represents the concentration of extract added and Y-axis represents the fold change over control of the mRNA expression of A549 cell lines

4. DISCUSSION

Many commercially proven anti cancer drugs used in modern times were initially used in crude form in traditional or folk healing practises or for other purposes [17,18]. An estimate states that 75% of 120 active plant derived compounds currently used worldwide have been through follow up studies to verify authenticity of data from folk and ethnomedical uses [19]. There is a great potential in discovery of traditional plant uses [20]. The extract with one of its phytochemicals called lupeol has increased the era gene expression in MDA – MB-231 (Era - negative breast cancer cells) thus inhibiting cell proliferation.

Plant phenols may be considered as potential compounds for selective blocking signal transduction pathways, this is in accordance with the current study where the *Aegle marmelos* blocks the MAPK pathway which inhibits the maturation of the Wnt-mRNA and beta-catenin mRNA [21-23]. The phytochemical profile of *Aegle marmelos* show pharmacological activity and hepatoprotective activity [24,6].

Derivatives of *Aegle marmelos* were found to be exhibiting strong activity in inhibiting *in vitro* cell growth of human K562 cells [25,26].

Aegle marmelos important pharmacological activities such as antidiabetic, antioxidant, antimicrobial, hepato-protective, anticancer and anti proliferative activity [27,28].

Extensive experimental and clinical studies prove that *Aegle marmelos* possess various properties which helped to play a role in prevention and treatment of many diseases [29].

The hydro alcoholic extract of *Aegle marmelos* exhibits strong antitumor and antioxidant activities on DLA-bearing mice [30,31].

Plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems [32,33]. *Aegle marmelos* has properties that are medicinal and is a hypoglycaemic extract [34,35].

Limitations of this study is, only when the study is done in in vivo conditions, exact results could be achieved but before advancements are needed to be developed , various steps of drug testing need to be approved. *In vitro* results have proven to be positive and this study also suggests a future study concentrating on the high significance of the extract against *Aegle marmelos*.

5. CONCLUSION

Hence, taking the entire result parameters into account, it can be concluded that *Aegle*

marmelos hydroethanolic leaf extract possesses chemotherapeutic potential by suppressing the lung tumour growth rate. It also has hepato-renal protective effects, and thus can be targeted as a novel and safe anticancer drug against lung cancer. Hence some of the extracts of the compound,if structurally identified and characterized, may be a potent candidate for anticancer drug development.

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 AND ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors would like to thank the Department of Physiology and Blue Lab for their participation for their kind cooperation throughout the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gowhari Shabgah A, Ezzatifar F, Aravindhan S, Olegovna Zekiy A, Ahmadi M, Gheibihayat SM, et al. Shedding more light on the role of Midkine in hepatocellular carcinoma: New perspectives on diagnosis and therapy. IUBMB Life. 2021;73(4):659–69.
2. J PC, Marimuthu T, C K, Devadoss P, Kumar SM. Prevalence and measurement of anterior loop of the mandibular canal using CBCT: A cross sectional study. Clin Implant Dent Relat Res. 2018;20(4): 531–4.
3. Masters J, Palsson BØ. Cancer Cell Lines. Springer Science & Business Media. 2006;377.
4. Barabadi H, Mojab F, Vahidi H, Marashi B, Talank N, Hosseini O, et al. Green synthesis, characterization, antibacterial and biofilm inhibitory activity of silver nanoparticles compared to commercial silver nanoparticles [Internet]. Inorganic Chemistry Communications. 2021;129: 108647. Available:http://dx.doi.org/10.1016/j.inoche.2021.108647
5. Ezhilarasan D. Critical role of estrogen in the progression of chronic liver diseases [Internet]. Hepatobiliary & Pancreatic Diseases International. 2020;19:429–34. Available:http://dx.doi.org/10.1016/j.hbpd.2020.03.011
6. Wadhwa R, Paudel KR, Chin LH, Hon CM, Madheswaran T, Gupta G, et al. Anti-inflammatory and anticancer activities of Naringenin-loaded liquid crystalline nanoparticles *in vitro*. J Food Biochem. 2021;45(1):e13572.
7. Wahab PUA, Madhulaxmi M, Senthilnathan P, Muthusekhar MR, Vohra Y, Abhinav RP. Scalpel versus diathermy in wound healing after mucosal incisions: A split-mouth study. J Oral Maxillofac Surg. 2018;76(6):1160–4.
8. McArthur HL, Hudis CA, Clinical Research Fellow, Breast Cancer Medicine Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, et al. Adjuvant Chemotherapy for Early-stage Breast Cancer [Internet]. Oncology & Hematology Review (US). 2007;57. Available:http://dx.doi.org/10.17925/ohr.2007.00.2.57
9. Ashraf SQ. Mechanisms of Antibody-based Killing of Colorectal Cancer Cell Lines; 2009.
10. Bharath B, Perinbam K, Devanesan S, AlSalhi MS, Saravanan M. Evaluation of the anticancer potential of Hexadecanoic acid from brown algae Turbinaria ornata on HT–29 colon cancer cells [Internet]. Journal of Molecular Structure. 2021; 1235:130229. Available:http://dx.doi.org/10.1016/j.molstruc.2021.130229
11. Kamath SM, Manjunath Kamath S, Jaison D, Rao SK, Sridhar K, Kasthuri N, et al. *In vitro* augmentation of chondrogenesis by Epigallocatechin gallate in primary Human chondrocytes - Sustained release model for cartilage regeneration [Internet]. Journal of Drug Delivery Science and Technology. 2020;60:101992.

Available:http://dx.doi.org/10.1016/j.jddst.2020.101992
12. Egbuna C, Mishra AP, Goyal MR. Preparation of phytopharmaceuticals for the management of disorders: the development of nutraceuticals and traditional medicine. Academic Press. 2020;574.
13. Jatav S, Dwivedi P, Singh M, Sehra N, Mishra BB. Properties and important molecules of medicinal interest in wood apple (*Aegle marmelos*) [Internet]. Synthesis of Medicinal Agents from Plants. 2018;127–50.
Available:http://dx.doi.org/10.1016/b978-0-08-102071-5.00006-4
14. Choudhary P. Evaluation of *Aegle marmelos* Extracts for Antibacterial, Antifungal, Antioxidant and Cytotoxic Properties [Internet]. Current Trends in Biomedical Engineering & Biosciences. 2017;9.
Available:http://dx.doi.org/10.19080/ctbeb.2017.09.555766
15. Pynam H, Dharmesh SM. Antioxidant and anti-inflammatory properties of marmelosin from Bael (*Aegle marmelos* L.); Inhibition of TNF-α mediated inflammatory/tumor markers. Biomed Pharmacother. 2018; 106:98–108.
16. Clarizia G, Bernardo P. Diverse Applications of Organic-Inorganic Nanocomposites: Emerging Research and Opportunities: Emerging Research and Opportunities. IGI Global. 2019;237.
17. Mudigonda SK, Murugan S, Velavan K, Thulasiraman S, Krishna Kumar Raja VB. Non-suturing microvascular anastomosis in maxillofacial reconstruction- a comparative study. J Craniomaxillofac Surg. 2020; 48(6):599–606.
18. Nambi G, Kamal W, Es S, Joshi S, Trivedi P. Spinal manipulation plus laser therapy versus laser therapy alone in the treatment of chronic non-specific low back pain: a randomized controlled study. Eur J Phys Rehabil Med. 2018;54(6):880–9.
19. Santhakumar P, Roy A, Mohanraj KG, Jayaraman S, Durairaj R. Ethanolic Extract of Capparis decidua Fruit Ameliorates Methotrexate-Induced Hepatotoxicity by Activating Nrf2/HO-1 and PPARγ Mediated Pathways [Internet]. Indian Journal of Pharmaceutical Education and Research. 2021;55:s265–74.
Available:http://dx.doi.org/10.5530/ijper.55.1s.59
20. Costa-Lotufo LV, Khan MTH, Ather A, Wilke DV, Jimenez PC, Pessoa C, et al. Studies of the anticancer potential of plants used in Bangladeshi folk medicine. J Ethnopharmacol. 2005;99(1):21–30.
21. Hathway DE. Plant phenols and tannins [Internet]. Chromatography. 1969;390–436.
Available:http://dx.doi.org/10.1016/b978-0-433-30503-3.50024-9
22. Prakash AKS, Devaraj E. Cytotoxic potentials of S. cumini methanolic seed kernel extract in human hepatoma HepG2 cells [Internet]. Environmental Toxicology. 2019;34:1313–9.
Available:http://dx.doi.org/10.1002/tox.22832
23. Rajakumari R, Volova T, Oluwafemi OS, Rajesh Kumar S, Thomas S, Kalarikkal N. Grape seed extract-soluplus dispersion and its antioxidant activity [Internet]. Vol. 46, Drug Development and Industrial Pharmacy. 2020;1219–29.
Available:http://dx.doi.org/10.1080/03639045.2020.1788059
24. Rathee P, Kamboj A, Sidhu S. Optimization and development of Nisoldipine nano-bioenhancers by novel orthogonal array (L27 array). Int J Biol Macromol. 2016;86:556–61.
25. Lambertini E, Piva R, Khan MTH, Lampronti I, Bianchi N, Borgatti M, 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. Int J Oncol. 2004;24(2):419–23.
26. Tahmasebi S, Qasim MT, Krivenkova MV, Zekiy AO, Thangavelu L, Aravindhan S, et al. The effects of oxygen-ozone therapy on regulatory T-cell responses in multiple sclerosis patients. Cell Biol Int. 2021;45(7):1498–509.
27. Sharma N, Janwal A, Reena, Janwal S. Bael- a tree with multiple uses [Internet]. Journal of Hill Agriculture. 2017;8:130.
Available:http://dx.doi.org/10.5958/2230-7338.2017.00025.8
28. Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. J Oral Pathol Med. 2019;48(4):299–306.
29. Rahman S, Quader MR, Khan MI. Prevention of peptic ulcer by aqueous extract of *Aegle marmelos* leaf in rats

[Internet]. IMC Journal of Medical Science. 2018;12:11–4. Available:http://dx.doi.org/10.3329/imcjms.v12i1.35171

30. Akhouri V, Kumari M, Kumar A. Therapeutic effect of *Aegle marmelos* fruit extract against DMBA induced breast cancer in rats [Internet]. Scientific Reports. 2020;10. Available:http://dx.doi.org/10.1038/s41598-020-72935-2
31. R H, Ramani P, Ramanathan A, R JM, S G, Ramasubramanian A, et al. CYP2 C9 polymorphism among patients with oral squamous cell carcinoma and its role in altering the metabolism of benzo[a]pyrene. Oral Surg Oral Med Oral Pathol Oral Radiol. 2020;130(3):306–12.
32. Saraswathi I, Saikarthik J, Senthil Kumar K, Madhan Srinivasan K, Ardhanaari M, Gunapriya R. Impact of COVID-19 outbreak on the mental health status of undergraduate medical students in a COVID-19 treating medical college: a prospective longitudinal study. Peer J. 2020;8: e10164.
33. Vivekanandhan K, Shanmugam P, Barabadi H, Arumugam V, Raj DDRD, Sivasubramanian M, et al. Emerging Therapeutic Approaches to Combat COVID-19: Present Status and Future Perspectives [Internet]. Frontiers in Molecular Biosciences. 2021;8. Available:http://dx.doi.org/10.3389/fmolb.2021.604447
34. Mudi SR, Akhter M, Biswas SK, Muttalib MA, Choudhury S, Rokeya B, et al. Effect of aqueous extract of *Aegle marmelos* fruit and leaf on glycemic, insulinemic and lipidemic status of type 2 diabetic model rats. J Complement Integr Med [Internet]. 2017;14(2). Available:http://dx.doi.org/10.1515/jcim-2016-0111
35. Kumari KDKP, K D K. Hypoglycaemic, Anti-inflammatory and ATPase Effect of the Dried Flower Extracts of Aegle niarnielos (Bael Fruit) [Internet]. Available:http://dx.doi.org/10.31357/fmesphd.2014.00129

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/74399