



Effect of Kabasura Kudineer Extract on Inhibitory Kappa B Kinase Beta and m TOR mRNA Complex Expression in Lung Cancer Cells (A549 Cell)

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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

Background: Lung cancer has the highest mortality rate and highest rate of metastasis. Kabasura kudineer was used widely during the Covid period. The aim of the study is to find the effect of kabasura kudineer extract on inhibitory kappa B kinase beta and mTOR mRNA complex expression in lung cancer cells.

Materials and Methods: Cell viability test was done using MTT assay. mRNA expression of inhibitory kappa B Kinase and mTOR was done by real-time PCR. The data was analysed statistically by one way analysis of variance and Duncan multiple range test with graph prism version 5. $p < 0.05$ was considered significant.

Results: Kabasura kudineer caused a marked increase in cell death in dose dependent manner. At the end of 48 hours, maximum inhibition was at 300 and 400 $\mu\text{g/ml}$. Kabasura kudineer has inhibited the mRNA expression of inhibitory kappa B Kinase and mTOR in lung cancer cell lines (A549 cell).

Conclusion: The study indicates that kabasura kudineer extract has anti cancer activity on the lung cancer cell line (A549 cell).

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1. INTRODUCTION

Siddha medicines is a traditional system of medicine which is widely used by the South Indian population. It originated more than 10,000 years ago. Siddha medicine is used for various diseases like psoriasis, STD, diseases in liver and gastrointestinal tract, arthritis and urinary tract infections [1]. It believes that food is the principle medicine. The efficiency of the medicines have also been examined scientifically in respiratory and other ailments [2].

Kabasura Kudineer and Novel Herbal Preparation which are commonly used in treating viral infections and respiratory infections and will be effective against the pandemic novel coronavirus SARS-CoV-2 [3]. Kabasura kudineer is a part of siddha medicine which is extracted from herbs. It is mostly used for respiratory diseases. It is reported to boost up the humoral immunity in the body. It has many phytoconstituents [4]. Kabasura kudineer have many pharmacological activities such as anti asthmatic, anti oxidant, anti immuno modulatory activity. Recently kabasura kudineer is used in COVID - 19 because of its antioxidant property [4,5]. Due to strong therapeutic effects, the medicinal plants have been traditionally used to treat diseases [6-8].

It is a compound formulation consisting of fifteen herbal ingredients. It's commonly used for the treatment of fever with or without respiratory tract infection. It has been widely used during the epidemic of swine influenza as a prophylactic and media reports gave a rebirth to the present official Siddha formulation. Siddha medicinal preparations are classified as 32 internal and 32 external medicinal forms and choornam is one of the interior medicinal forms. The drug is further classified as kudineer choornam which suggests a drug to be made into liquid form and consumed [9]. Kabasura Kudineer is the name given to the Siddha formulation during which the entire plant or the actual part of the plant is grinded into powder. The obtained powder is named 'choornam'. It's then made into a kudineer by adding water and heated till water reduces to 1/4th or 1/8th of its volume. It's then filtered and filtrate is employed. Dose of the kudineer is usually 30 ml before food, three to fourfold each day. Lifetime of a prepared kudineer is 3 hours [10]. The way of preparation of kudineer is a simple process and therefore the

phytoconstituents doesn't undergo any major change while processing and preparation, unlike other traditional formulations.

Lung cancer carcinoma cases and deaths are rising [11]. In 2018, GLOBOCAN estimated 2.09 million new cases (11.6% of total cancer cases) and 1.76 million deaths (18.4% of total cancer deaths), above 2012 reported rates (1.8 million new cases and 1.6 million deaths), making it the foremost frequent cancer and explanation for cancer death in men and ladies combined and in women, the third common cancer type and therefore the second common explanation for cancer death [12].

Owing to the strong therapeutic effects, the medicinal plants are being traditionally used to treat several diseases [8] [13,14]. Different parts of medicinal plants have numerous nutraceutical values and are enriched with proteins, carbohydrates, vitamins, fibre, potassium, calcium and also the presence of phytoconstituents contributes to its significant medicinal property [15–18].

Between countries, significant variation in carcinoma incidence and demographic distribution are evaluated, and tobacco smoking rates and stage of economic development influence these patterns. Although cancer statistics in the developing countries are less reliable, carcinoma incidence is predicted to grow more in developing regions. Aim of the study is to evaluate the effect of Kabasura kudineer extract on inflammatory cytokines in the lung cancer cell line. Our team has extensive knowledge and research experience that has translated into high quality publications [19-36]. The ingredients of kabasura kudineer may have anti cancer effects, hence the study was intended to investigate its effect on lung cancer cells.

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 benzimidazolo carbocyanine iodide) and Real Time PCR kit was purchased TAKARA

(Meadowvale Blvd, Mississauga, ON L5N 5S2, Canada).

2.1 Cell Line and Cell Structure

Human lung cancer cell line (A549) was purchased from the National Centre for Cell Sciences (NCCS), Pune, India. Cells were cultured in DMEM 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% CO₂.

2.2 Cell Viability by MTT Assay

Cell viability was assayed using a modified colorimetric technique that is based on the ability 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 Kabasura kudineer extract (100-500µg/ml) with A549 cells for 48 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. Then the formazan crystals formed were dissolved in dimethyl sulfoxide (100 µl) and incubated at 37°C. Then the intensity of color which was developed is 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 extract free medium. In control medium cell viability without any treatment was represented as 100%. The cell viability is calculated by the formula: % cell viability = [A_{570 nm} of treated cells/A_{570 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 quality control purposes, for all samples melting curves were acquired. The specificity of the amplification product was determined by melting curve analysis for each primer pair. The data were analyzed by comparative CT method and the fold change is calculated by 2^{-ΔΔCT} method described by Schmittgen 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

3.1 Effect of Kabasura Kudineer on the Cell Viability

Kabasura kudineer was evaluated against lung cancer cell line (A549) and cell viability was determined after administering the different doses of kabasura kudineer, It was found to exhibit inhibition of cancer cells by decreasing the percent viability of cancer cells in dose dependent manner. It was found that maximum inhibition of cell growth was at maximum concentration (300-400µg/ml) used in this study when compared to control (Fig. 1).

3.2 Effect of Kabasura Kudineer on the mRNA Expression of Inhibitory Kappa B Kinase Beta

The activity of IKKB alpha was assessed in a dose dependent manner. In the treated cancer lines, There was no significant reduction in mRNA expressions of inhibitory kappa B kinase beta at 400 and 500µg/ml. (p>0.05) (Fig. 2).

3.3 Effect of Kabasura Kudineer on the mRNA Expressions of mTOR

The mRNA expression of mTOR was assessed in a dose dependent manner. The cancer cells were significantly inhibited and it was found that there was significant reduction in mRNA

expression of mTOR when compared to control interestingly, the decrease was in dose dependent manner [37] (Fig. 3). at a dose of 400 and 500 µg/ml ($p < 0.05$). Thus

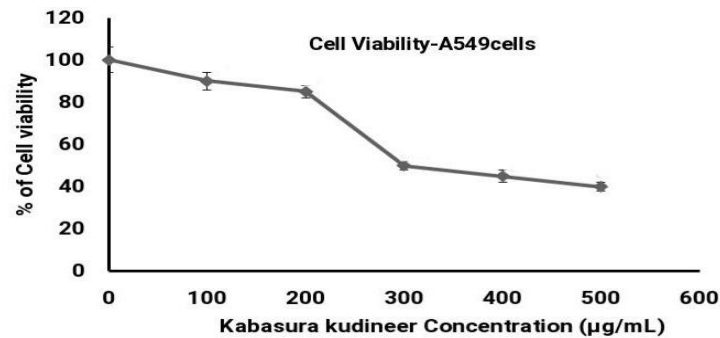


Fig. 1. shows the Effect of kabasura kudineer extract on cell viability in A549 cells. Each bar represents a mean \pm SEM of 6 observations. Significance at $p < 0.05$

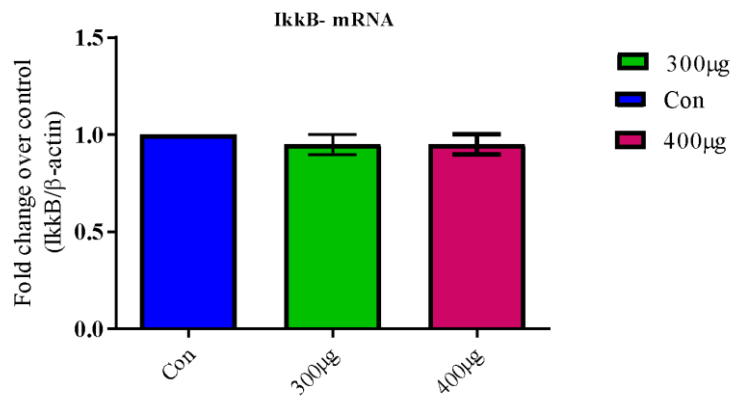


Fig. 2. Effect of kabasura kudineer extract on IL-6 mRNA expression in A549 cells. Each bar represents a mean \pm SEM of 6 observations. The X-axis represents - different concentrations of Kabasura kudineer and the Y axis represents the fold change over control. Statistically there was no significant difference between the control and treated groups ($p > 0.05$)

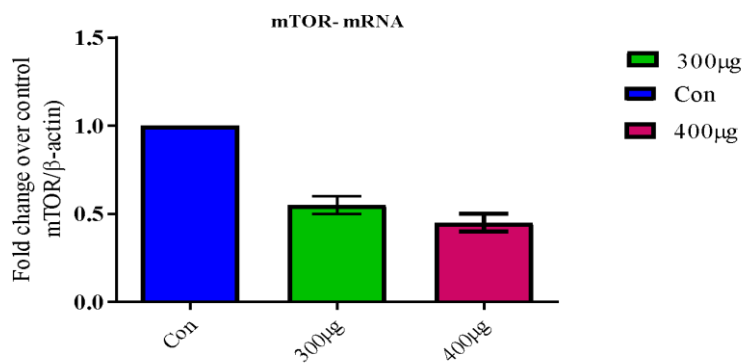


Fig. 3. Effect of kabasura kudineer extract on TNF alpha mRNA expression in A549 cells. Each bar represents a mean \pm SEM of 6 observations. The X-axis represents - different concentrations of Kabasura kudineer and the Y axis represents the fold change over control. a-compared with untreated control cells. There is a statistically significant difference between the control and treated groups ($p < 0.05$)

4. DISCUSSION

Kabasura kudineer can be used as a home remedy to boost up immunity and stop from viral infections. Kabasura kudineer is found to be more cost effective than allopathic medicine which may have side effects and used as a self medication to impress our body from resisting all kinds of viral and fungal infection [38]. People find that kabasura kudineer has less side effects compared to allopathic medicine since it's found to have natural products. The necessity to extend research where the efficiency, safety and potential of Siddha medicines are to be tested using clinical trials. Such studies will be a benchmark for other developing nations to enhance their stance within the health care sector [38,39]. The present study found that kabasura kudineer had a significant cytotoxic effect against lung cancer cell lines and is dose dependent at the concentration 300-400µg/ml. Previously the studies have been conducted on ginger, one of the components of kabasura kudineer and it was reported that it has an anticancer effect by inhibiting mTOR pathway and this is similar to our study [40].

NF- kappa B may be a mediator for carcinoma and is typically targeted for carcinoma prevention (Chen et al., 2011). Inhibitory kappa B kinase beta (IKK β / Ikk β) helps within the activation of the NF- κ B transcription factor family by the phosphorylation of I κ B inhibitors (Bai et al., 2011). The NF- κ B transcription factors help within the balance between cell survival and regulation of cell proliferation and differentiation of the many cell types. The changes within the activity of Ikk β and NF- κ B is claimed to be found in many diseases which include acute and chronic inflammation [41], mTOR (the mechanistic target of rapamycin) is a serine/threonine protein kinase in the PI3K based kinase [42]. The mTOR pathway integrates growth factor signals and nutritional status. The mTOR pathway is an intracellular signalling network which is often hyperactivated in many types of cancer. It is known to have prototypic functions in cellular proliferation, growth, differentiation and survival. Therefore, the mTOR signalling pathway is a promising tool against cancer [43]. This study shows that mTOR shows inhibitory properties when cancer cell lines were treated with kabasura kudineer. This is an in vitro study but to have a conclusive result further studies can be conducted in animal models. Further studies can be carried out in the future in

kabasura kudineer to exactly find out about the underlying mechanisms.

5. CONCLUSION

This study showed that kabasura kudineer has cytotoxic effect against lung cancer cell lines through inhibition of Inhibitory kappa B kinase beta and mTOR mRNA expression. This effect could be attributed to the presence of phytochemicals present in the 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.

NOTE

The study highlights the efficacy of "Siddha medicines" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. 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>
2. 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>
 3. Natarajan S, Anbarasi C, Sathiyarajeswaran P, Manickam P, Geetha S, Kathiravan R, et al. The efficacy of Siddha Medicine, Kabasura Kudineer (KSK) compared to Vitamin C & Zinc (CZ) supplementation in the management of asymptomatic COVID-19 cases: A structured summary of a study protocol for a randomised controlled trial. Trials. 2020; 21(1):892.
 4. Anbarasu K, Manisenthil KKT, Ramachandran S. Antipyretic, anti-inflammatory and analgesic properties of nilavembu kudineer choornam: a classical preparation used in the treatment of chikungunya fever [Internet]. Asian Pacific Journal of Tropical Medicine. 2011;4:819–23.
Available: [http://dx.doi.org/10.1016/s1995-7645\(11\)60201-0](http://dx.doi.org/10.1016/s1995-7645(11)60201-0)
 5. Bisignano G, Iauk L, Kirjavainen S, Galati EM. Anti-inflammatory, analgesic, antipyretic and antibacterial activity of *Astragalus siculus* Biv [Internet]. International Journal of Pharmacognosy. 1994;32:400–5. Available: <http://dx.doi.org/10.3109/13880209409083023>
 6. *In vitro* Antibacterial, Free Radical Scavenging activity of aqueous and ethanolic extracts of *Capparis decidua* [Internet]. International Journal of Pharmaceutical Research. 2020;12. Available:<http://dx.doi.org/10.31838/ijpr/2020.sp1.450>
 7. *In-vitro* Free Radical Scavenging, Antibacterial, Cytotoxicity of Ethanolic/Hydroethanolic Extract of *Capparis Decidua* [Internet]. International Journal of Pharmaceutical Research. 2020;12. Available:<http://dx.doi.org/10.31838/ijpr/2020.sp1.438>
 8. Lakshme PST, Preetha, Jeevitha M, Rajeshkumar S. Evaluation of antioxidant and Cytotoxic effect of selenium nanoparticles synthesised using *Capparis decidua*. J Pharm Res Int. 2020;60–6.
 9. Vincent S, Arokiyaraj S, Saravanan M, Dhanraj M. Molecular docking studies on the anti-viral effects of compounds from Kabasura Kudineer on SARS-CoV-2 3CL. Front Mol Biosci. 2020;7:613401.
 10. Shanmugasundaram N, Chandrasekaran A. A prospective, single-centre, randomized open labelled comparative clinical study to evaluate the effectiveness of Siddha medicine, Kabasura kudineer and vitamin c-zinc supplementation in the management of asymptomatic COVID 19 patients. v1 (protocols.io.biebkban) [Internet]. protocols.io. Available:<http://dx.doi.org/10.17504/protocols.io.biebkban>
 11. Roth JA, Cox JD, Hong WK. Lung Cancer. John Wiley & Sons. 2011;480.
 12. Monteiro L de S, Bastos KX, Barbosa-Filho JM, de Athayde-Filho PF, Diniz M de FFM, Sobral MV. Medicinal Plants and Other Living Organisms with Antitumor Potential against Lung Cancer. Evid Based Complement Alternat Med. 2014; 2014:604152.
 13. *In vitro* Antibacterial, Free Radical Scavenging Activity of Aqueous and Ethanolic Extracts of *Capparis decidua* [Internet]. International Journal of Pharmaceutical Research. 2020;12. Available:<http://dx.doi.org/10.31838/ijpr/2020.sp1.450>
 14. *In-vitro* free radical scavenging, antibacterial, cytotoxicity of ethanolic/hydroethanolic extract of *Capparis decidua* [Internet]. International Journal of Pharmaceutical Research. 2020;12. Available:<http://dx.doi.org/10.31838/ijpr/2020.sp1.438>
 15. Anushya PAP, Preetha S, Prathap L, Jeevitha M. Enhanced antibacterial activity of capparid fruit mediated selenium nanoparticle against *Enterococcus faecalis* [Internet]. International Journal of Research in Pharmaceutical Sciences. 2021;12:6–11. Available:<http://dx.doi.org/10.26452/ijrps.v12i1.3905>

16. Chockalingam S, Preetha S, Jeevitha M, Pratap L. Antibacterial effects of *Capparis decidua* fruit mediated selenium nanoparticles [Internet]. Journal of Evolution of Medical and Dental Sciences. 2020;12:2947–50. Available:<http://dx.doi.org/10.14260/jemds/2020/646>
17. Janani KJ, Preetha S, Jeevitha, Rajeshkumar S. Green synthesis of selenium nanoparticles using *Capparis decidua* and its anti-inflammatory activity [Internet]. International Journal of Research in Pharmaceutical Sciences. 2020;11:6211–5. Available:<http://dx.doi.org/10.26452/ijrps.v11i4.3298>
18. Ali SJ, Preetha S, Jeevitha M, Prathap L, S. R. Antifungal activity of selenium nanoparticles extracted from *Capparis decidua* fruit against *Candida albicans* [Internet]. Journal of Evolution of Medical and Dental Sciences. 2020;9:2452–5. Available:<http://dx.doi.org/10.14260/jemds/2020/533>
19. 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. PeerJ. 2020;8:e10164.
20. 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 PPAR γ mediated pathways. Ind J Pharm Educ. 2021;55(1s):s265–74.
21. 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.
22. Rajakumari R, Volova T, Oluwafemi OS, Rajesh Kumar S, Thomas S, Kalarikkal N. Grape seed extract-soluplus dispersion and its antioxidant activity. Drug Dev Ind Pharm. 2020;46(8):1219–29.
23. Clarizia G, Bernardo P. Diverse applications of organic-inorganic nanocomposites: Emerging research and opportunities: Emerging research and opportunities. IGI Global. 2019;237.
24. 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>
25. 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.
26. 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.
27. 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>
28. Ezhilarasan D. Critical role of estrogen in the progression of chronic liver diseases. Hepatobiliary Pancreat Dis Int. 2020;19(5):429–34.
29. 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.
30. 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>
31. 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.
32. 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.
33. R H, Hannah R, Ramani P, Ramanathan A, Jancy MR, Gheena S, et al. CYP2 C9 polymorphism among patients with oral squamous cell carcinoma and its role in altering the metabolism of benzo[a]pyrene [Internet]. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology.* 2020; 130:306–12. Available:<http://dx.doi.org/10.1016/j.oooo.2020.06.021>
34. J PC, Pradeep CJ, Marimuthu T, Krithika C, Devadoss P, Kumar SM. Prevalence and measurement of anterior loop of the mandibular canal using CBCT: A cross sectional study [Internet]. *Clinical Implant Dentistry and Related Research.* 2018;20:531–4. Available:<http://dx.doi.org/10.1111/cid.12609>
35. Wahab PUA, Abdul Wahab PU, Madhulaxmi M, Senthilnathan P, Muthusekhar MR, Vohra Y, et al. Scalpel versus diathermy in wound healing after mucosal incisions: A split-mouth study [Internet]. *Journal of Oral and Maxillofacial Surgery.* 2018;76:1160–4. Available:<http://dx.doi.org/10.1016/j.joms.2017.12.020>
36. Mudigonda SK, Murugan S, Velavan K, Thulasiraman S, Krishna Kumar Raja VB. Non-suturing microvascular anastomosis in maxillofacial reconstruction- A comparative study. *Journal of Cranio-Maxillofacial Surgery.* 2020;48(6):599–606.
37. Hua H, Kong Q, Zhang H, Wang J, Luo T, Jiang Y. Targeting mTOR for cancer therapy. *J Hematol Oncol.* 2019;12(1):71.
38. K G, Gopalasatheeskumar K, Lakshmanan K, Moulishankar A, Suresh J, Kalaiichelvan VK, et al. Screening of kabasura kudineer chooranam against COVID-19 through targeting of main protease and RNA-Dependent RNA Polymerase of SARS-CoV-2 by Molecular Docking Studies [Internet]. *SSRN Electronic Journal.* Available:<http://dx.doi.org/10.2139/ssrn.3625653>
39. Kiran G, Karthik L, Shree Devi MS, Sathiyarajeswaran P, Kanakavalli K, Kumar KM, et al. In Silico computational screening of Kabasura Kudineer - official siddha formulation and JACOM against SARS-CoV-2 spike protein [Internet]. *Journal of Ayurveda and Integrative Medicine;* 2020. Available:<http://dx.doi.org/10.1016/j.jaim.2020.05.009>
40. Paul A, Edwards J, Pepper C, Mackay S. Inhibitory- κ B Kinase (IKK) α and Nuclear factor- κ B (NF κ B)-Inducing Kinase (NIK) as Anti-Cancer Drug Targets. *Cells* [Internet]. 2018;7(10). Available:<http://dx.doi.org/10.3390/cells7100176>
41. Sughra K, Birbach A, de Martin R, Schmid JA. Interaction of the TNFR-receptor associated factor TRAF1 with I-kappa B kinase-2 and TRAF2 indicates a regulatory function for NF-kappa B signaling. *PLoS One.* 2010;5(9):e12683.
42. Zhou M, Shen S, Zhao X, Gong X. Luteoloside induces G/G arrest and pro-death autophagy through the ROS-mediated AKT/mTOR/p70S6K signalling pathway in human non-small cell lung cancer cell lines. *Biochem Biophys Res Commun.* 2017;494(1-2):263–9.
43. Liu F, Gao S, Yang Y, Zhao X, Fan Y, Ma W, et al. Antitumor activity of curcumin by modulation of apoptosis and autophagy in human lung cancer A549 cells through inhibiting PI3K/Akt/mTOR pathway. *Oncol Rep.* 2018;39(3):1523–31.

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