CARAS
© 2023, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 14; Issue 05 (Sep-Oct 2023); pp 1112–1117

Open Access
ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Full Length Research Article

Evaluation of the Anti-Cancer Potential of *Aloe vera* and *Terminalia chebula* Herbal Formulation against MCF-7 Breast Cancer Cell Line and Characterization of Silver Nanoparticles of the Herbal Formulation

P. G. Geegi*[1], A. Benno Susai Vijayakumar[1], T. Antony Diwakar Chandran[1], Asha Monica A[2], P. Elakkiya[3] and T. Prabhash[4]

[1] Department of Biochemistry, St. Joseph's College (Autonomous) Affiliated to Bharathidasan University, Trichy - 620 002, Tamil Nadu, India
[2] Department of Biotechnology, St. Joseph's College (Autonomous) Affiliated to Bharathidasan University, Trichy - 620 002, Tamil Nadu, India
[3] Rajas Dental College and Hospital, Kavalkinaru Jn, Tirunelveli - 627105, Tamil Nadu, India
[4] Department of Biochemistry, Bharathidasan University, Trichy - 620 024, Tamil Nadu, India

Abstract

Traditional Medicinal System (TMS) has been practiced for centuries and is a longstanding companion in the fight against diseases. India possesses the Indian System of Medicines (ISM), which includes Ayurveda, Siddha, Unani, Naturopathy, and Homoeopathy. *Aloe vera* and *Terminalia chebula* herbal formulation are traditional herbs used for various ailments. This study focuses on the synthesis and characterization of silver nanoparticles (AgNPs) derived from the *Aloe vera* and *Terminalia chebula* herbal formulation. Silver nitrate and leaf aqueous extracts were used as a precursor and capping reducing agent, respectively. The AgNPs were characterized using UV-Visible Spectrophotometer (UV), Particle size Analyzer (DLS), Scanning electron microscope (SEM), and Fourier-transform infrared spectroscopy (FT-IR). Furthermore, the anti-carcinogenic potential of the *Aloe vera* and *Terminalia chebula* herbal formulation against the MCF-7 cell line was evaluated through in vitro analysis.

***Key words*:** *Terminalia chebula*, Ayurveda, Siddha, Unani, Naturopathy, Homoeopathy

Traditional medicine systems have been utilized by indigenous peoples worldwide, with Chinese, Indian, and African systems being particularly renowned [26], [30]. Siddha medicine, one of the oldest medical systems in India, has flourished in the southern part of the country, especially in Tamil Nadu [1]. Siddha and Ayurveda are the two most ancient medical systems in India. Siddha, the mother medicine of ancient Tamils/Dravidians of South India, was formerly known as Marunthu (Medicine) [2]. Siddha formulations are believed to act on multiple biochemical pathways and can influence several organ systems simultaneously, providing nutrition and reducing the side effects of modern therapeutic interventions. Breast cancer is a significant and life-threatening disease that continues to increase in frequency globally. Aloe vera, also known as *Aloe barbadensis* or Aloe vera, has a long history of use in topical and oral therapeutics. It is a hardy perennial tropical plant belonging to the Liliaceae family, with a wide range of medicinal applications in indigenous systems of medicine such as Ayurveda, Siddha, Unani, and Homeopathy [24]. *Terminalia chebula* is a highly branched deciduous tree with various pharmacological activities, including hypolipidemic, hypocholesterolemic, and anti-spermatogenic properties. Nanoparticle biosynthesis involves reduction and oxidation processes, and scientists have used microbial enzymes and plant extracts for synthesizing nanoparticles. Green synthesis of nanoparticles is cost-effective, environmentally friendly, and allows for easy large-scale synthesis without the need for high temperature, pressure, or toxic chemicals [27].

In recent decades, natural medicine has experienced exponential growth in popularity. Medicines derived from natural plant sources are gaining traction in both developing and developed countries due to their natural origin and lower incidence of side effects [25]. Natural drugs form a significant portion of India's officially recognized systems of health, such as Ayurveda, yoga, unani, siddha, homeopathy, and naturopathy, with more than 70% of India's population still relying on these non-allopathic systems of medicine [3]. Natural drugs are considered safe and easily accessible. The aim of this study is to evaluate the anti-tumor potential of the *Aloe*

Received: 01 Jun 2023; Revised accepted: 12 July 2023; Published online: 02 Sep 2023

Correspondence to: P. G. Geegi, Department of Biochemistry, St. Joseph's College (Autonomous) Affiliated to Bharathidasan University, Trichy - 620 002, Tamil Nadu, India; E-mail: ashamonica7@gmail.com

Citation: Geegi PG, Vijayakumar ABS, Chandran TAD, Monica AA, Elakkiya P, Prabhash T. 2023. Evaluation of the anti-cancer potential of *Aloe vera* and *Terminalia chebula* herbal formulation against MCF-7 breast cancer cell line and characterization of silver nanoparticles of the herbal formulation. *Res. Jr. Agril. Sci.* 14(5): 1112-1117.

vera and *Terminalia chebula* herbal formulation against breast cancer cell lines (MCF-7) [23], [28].

MATERIALS AND METHODS

Collection of plant materials: The herbal formulation consisted of *Aloe vera* and *Terminalia chebula* fruit powder. Aloe vera plants were collected from the Manachanallur area, Tiruchirapalli, while *Terminalia chebula* fruit powder was obtained from the Apollo Pharmacy in Tiruchirapalli.

Preparation of herbal formulation: The herbal formulation was prepared by mixing *Aloe vera* pulp, obtained by peeling and washing the *Aloe vera* plant, with *Terminalia chebula* fruit powder. The mixture was allowed to stand for 1-2 hours, filtered, and reduced by decoction to obtain the final herbal formulation [4], [20].

Synthesis of silver nanoparticles: Silver nanoparticles were synthesized by mixing silver nitrate with the herbal formulation and incubating the mixture overnight. After centrifugation, the pellet was dissolved in distilled water, and the synthesized nanoparticles were characterized using UV-Visible Spectroscopy, FT-IR, SEM, and particle analyzer [13-15].

Evaluation of cytotoxicity of herbal formulation: Human breast cancer cell line MCF-7 was used for in vitro analysis [8], [19]. Cytotoxicity assays were performed using the trypan blue exclusion method, MTT assay, and lactate dehydrogenase (LDH) release assay [29].

RESULTS AND DISCUSSION

Phytochemical screening of the *Aloe vera* and *Terminalia chebula* herbal formulation revealed the presence of flavonoids, saponins, steroids, carbohydrates, tannins, and terpenoids, which may contribute to its cytotoxic potential [5]. The synthesized silver nanoparticles were characterized using UV-Visible spectroscopy, FT-IR, SEM, and particle analyzer, confirming their successful formation and providing information about their size and morphology. The cytotoxicity evaluation of the herbal formulation against the MCF-7 cell line showed a dose-dependent decrease in cell viability in all three assays, indicating its potential as an anti-cancer agent [6].

Table 1 Result of phytochemical screening of *Aloe vera* and *Terminalia chebula* herbal formulation

S. No.	Test	Inference
1.	Alkaloids	-
2.	Flavonoids	+
3.	Saponins	+
4.	Steroids	+
5.	Carbohydrates	+
6.	Glycosides	-
7.	Proteins	-
8.	Phenols	-
9.	Tannins	+
10	Anthraquinone	-
11	Terpenoids	-

Data depicted in (Table 1, Fig 1a-b) show the presence of flavonoids, saponins, steroids, carbohydrates, tannins and Terpenoids which might be responsible for its cytotoxic potential of the herbal formulation.

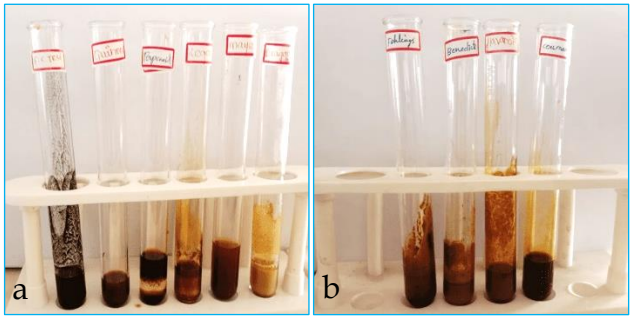

Fig 1a-b Result of the phytochemical screening of *Aloe vera* and *Terminalia chebula* herbal formulation

Characterization of silver nanoparticles

UV-Visible spectroscopy

In the present study, The UV–VIS absorption spectra of AgNPs were given in figure 1. It shows a peak at 215.6 and 269.9 nm which is attributed to the formation of silver nanoparticles which is in close agreement with the earlier reports [17].

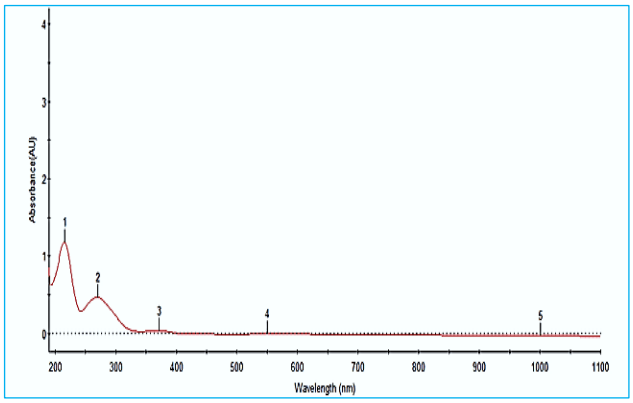

Fig 2 UV- Visible spectra of silver nanoparticles of herbal formulation of *Aloe vera* and *Terminalia chebula*

FTIR characterization

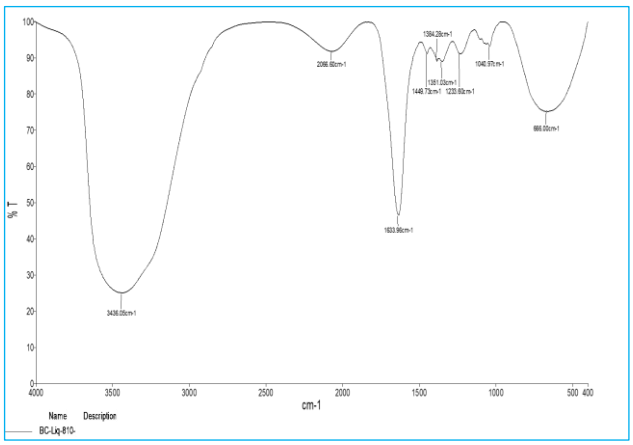

Fig 3 FTIR spectra of Herbal formulation of *Aloe vera and Terminalia chebula* silver nanoparticles

The FTIR measurements for the synthesized silver NPs is given in (Fig 2) which helps in identifying possible biomolecules that acts as reducing and capping agents. The strong peaks observed at 3436, 2066, 1633 and 1384 cm^{-1}corresponds to OH, N≡C C=C and aliphatic C–H stretching vibrations respectively. The peaks at 666 cm^{-1} corresponds to silver nanoparticles [18].

Scanning electron microscopy

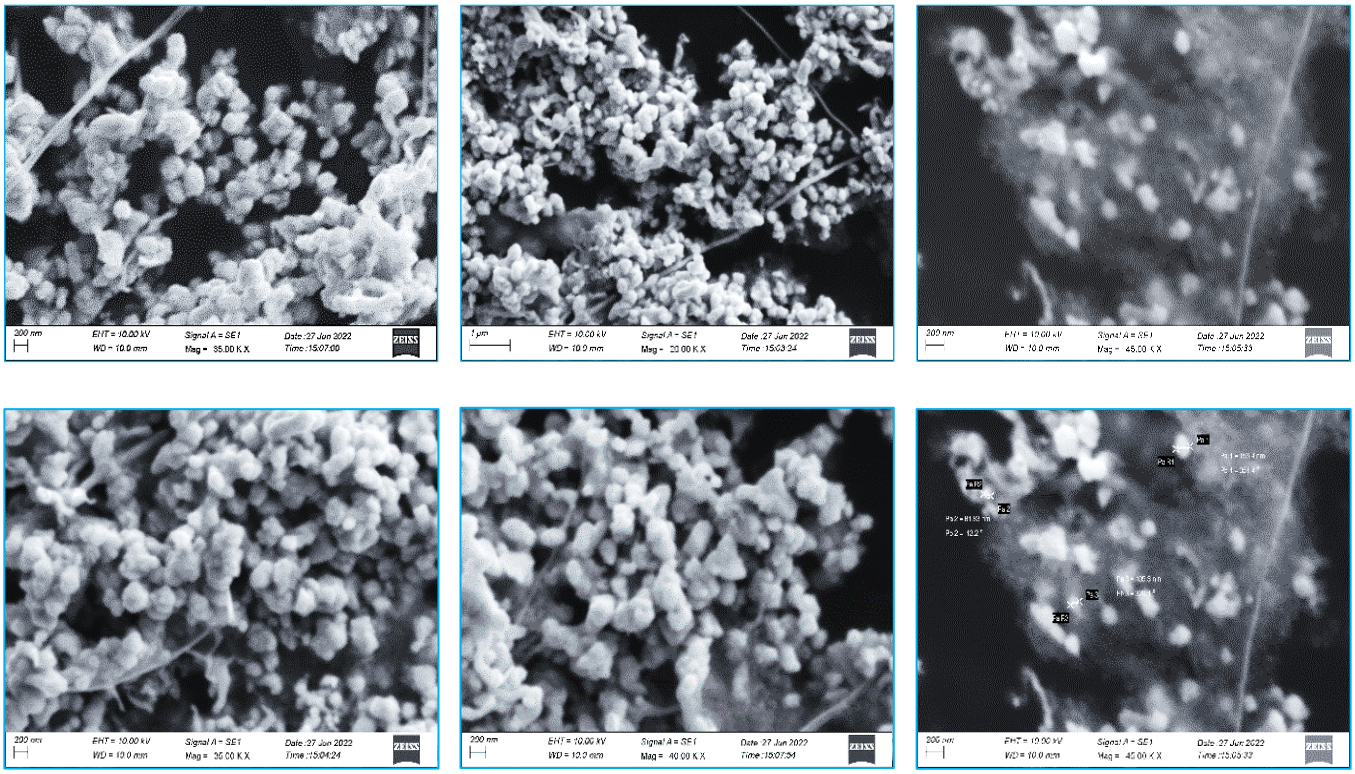

Fig 4 SEM images of silver nanoparticles

The surface morphology the silver NPs synthesized using Herbal formulation of *Aloe vera* and *Terminalia chebula* was shown in (Fig 4). It shows the presence of nanoparticles with more or less uniform shape but varying in sizes.

Particle analyzer

Assessed the hydrodynamic diameters of AgNPs and found that the cumulant diameters of AgNPs suspended in H_2O was 315nm shows in (Fig 4).

Anti-cancer activity of herbal formulation

Tryphan blue exclusion method

To assess time dependent cytotoxicity, direct counting of viable and non-viable cells after exposure to Herbal formulation of *Aloe vera and Terminalia chebula* against MCF-7 cell line were carried out using tryphan blue dye assay [7]. Untreated cells served as control. Resultant cell suspension was then admixed with 0.2% tryphan blue dye and counted in haemocytometer [10].

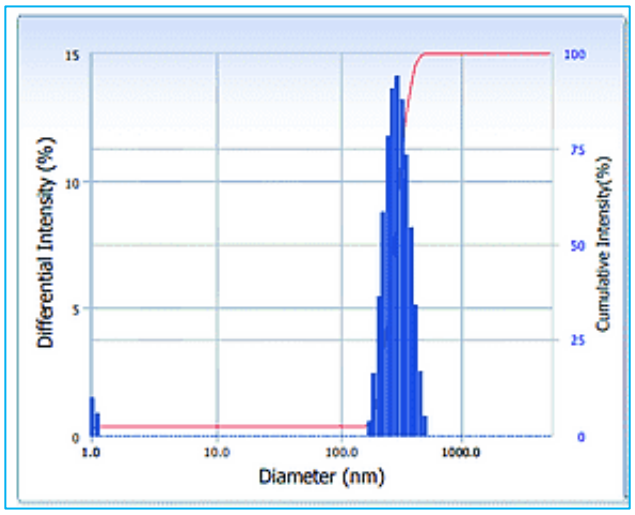

Fig 5 AgNPs hydrodynamic particle sizes in H_2O were also assessed by a Beckman Coulter Delsa Nano particle analyzer

Table 2 Cytotoxicity potential of *Aloe vera* and *Terminalia chebula* MCF-7 Cell lines -Tryphan blue exclusion method

Concentration (µg)	Dead cells (%)
Control	4.68 ± 1.26
100	16.86 ± 1.54
200	28.43 ± 2.02
300	36.68 ± 0.42
400	50.72 ± 2.09
500	69.74 ± 1.28

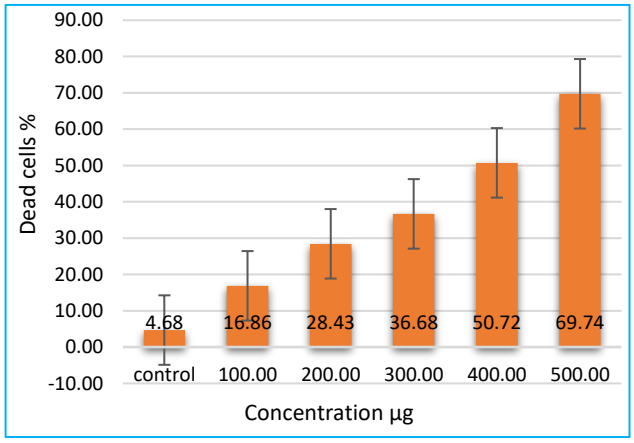

Fig 6 Cytotoxicity potential of *Aloe vera* and *Terminalia chebula* herbal formulation against MCF-7 Cell lines -Trypan blue exclusion method

(Table 2, Fig 6) shows that MCF-7 cell lines treated with various concentrations of *Aloe vera and Terminalia chebula* herbal formulation (100, 250, 500, 750 and 1000 µg/ml) showed progressive reduction in cell viability over a period of 3 hrs. In fact, at 3hrs there was a complete loss of cell viability in all the cancer cell lines with treatment of herbal formulation against MCF-7, the percentage of non-viability cells at 0, 100, 250, 500, 750 and 1000 µg/ml were found to be 4.5%, 16.86%, 28.43%, 36.68%, 50.72%, and 69.74% respectively. IC_{50} of herbal formulation was analyzed and noted as 500 µg/ml. In this

study, the cytotoxicity effect of Herbal formulation was more than 50% at maximum concentration. It indicates that the herbal formulation has effective and high anticancer activity. Tryphan blue, a diazo dye, is a vital stain used to selectively colour dead tissues or cells blue. Breast cells or tissues with intact cell membranes are not coloured. Since cells are very selective in the compounds that pass through the membrane, in a viable cell, tryphan blue is not absorbed; however, its transverses the membrane in a dead cell. Hence, nonviable cells are excluded from this staining and this aglycones could provide the chemical basis of its staining method described as a dye exclusion cytotoxic effect against MCF-7 cell line. From the results of tryphan blue dye exclusion method, it was observed that the cytotoxic effect of Herbal formulation was increased with increase in the concentration. It was found that it has effective cytotoxic potential against MCF-7 cell line [9] [12].

MTT Assay

Table 3 Cytotoxic potential of herbal formulation against MCF-7 cell lines -MTT assay method

Concentration (µg)	Cytotoxicity (%)
15.62	8.50 ± 0.53
31.25	16.83 ± 1.31
62.50	35.80 ± 0.53
125.00	47.43 ± 0.63
250.00	66.43 ± 0.56

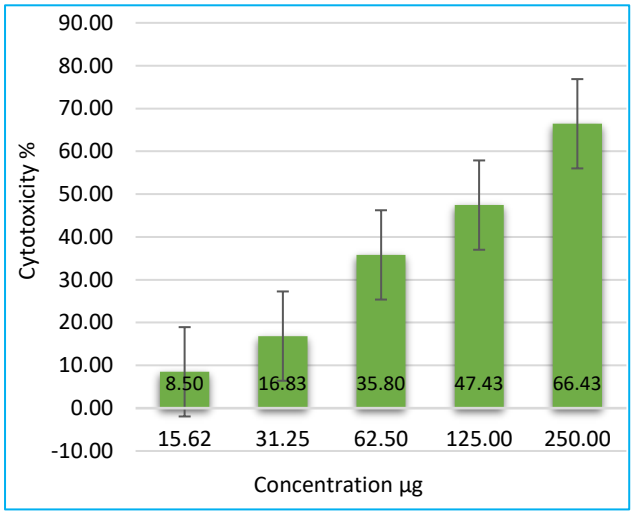

Fig 7 Cytotoxicity potential of *Aloe vera* and *Terminalia chebula* Herbal formulation against MCF-7 Cell lines -MTT

Assay method

In this study, MCF-7 cell line was treated with *Aloe vera and Terminalia chebula* Herbal formulation at different concentration for 24h and 48h [35]. A mitochondrial enzyme in living cells, succinate dehydrogenase cleaves the tetrazolium ring of MTT and converting it to an insoluble purple formazan. Therefore, the amount of formazon produced is directly proportional to the number of viable cells [30]. Viability of the MCF-7 cancer cells was decreased with increasing concentration of Herbal formulation (15.62 to 2250 µg/ml). Thus, the herbal formulation exhibited a high cell inhibition rate against MCF-7 as 8.50, 16.83, 35.80, 47.43 and 66.43% respectively. IC 50 value for MCF-7 was found to be 250 µg/ml.

The results of tryphan blue exclusion method are in agreement with the IC50 values calculated in the MTT assay. Both cytotoxic assays presented have comparable results and the small differences was observed in the cytotoxic values might be due to their assay principles, MTT reduction assessing the functional metabolic activity of mitochondria based on the enzymatic reduction of a tetrazolium salt by mitochondrial dehydrogenases of viable cells, while tryphan blue is based on cell membrane integrity [34].

LDH Assay method

Table 4 Cytotoxicity potential of *Aloe vera* and *Terminalia Chebula* against MCF-7 Cell lines -LDH assay method

Concentration (µg)	Cytotoxicity (%)
15.62	10.23±0.7
31.25	18.37±0.85
62.50	28.43±0.66
125.00	47.43±0.50
250.00	62.93±0.50

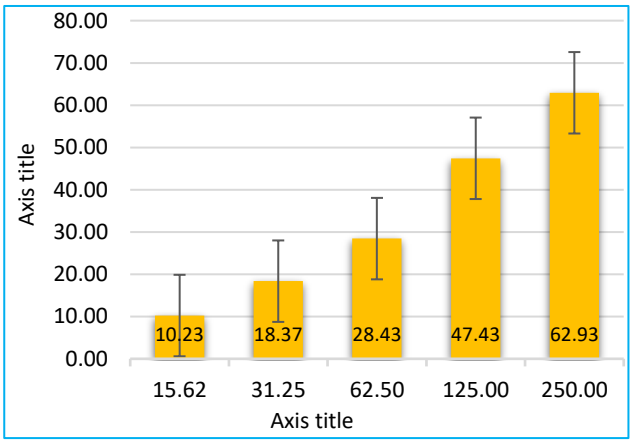

Fig 8 Cytotoxicity potential of *Aloe vera* and *Terminalia chebula* against MCF-7 Cell lines -LDH Assay method

The kinetic determination of the amount of the enzyme lactate dehydrogenase (LDH) released from lysed target cells was determined to provide a sensitive and precise measurement of natural cytotoxicity when used in conjunction with appropriate controls and calculational methods. LDH is oxidoreductase present in a wide variety of organisms. It catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD [33]. When disease or injury or toxic material damages tissues, cells release LDH into the bloodstream. Since LDH is a fairly stable enzyme, LDH has been widely used to evaluate the presence of damage and toxicity of tissue and cells. Quantification of LDH has a broad range of applications. In this colorimetric LDH quantification assay, LDH reduces NAD to NADH, which then interacts with a specific probe to produce a colour (λmax = 340 nm) [32]. *Aloe vera and Terminalia chebula* showed the 10.23, 18.37, 28.43, 47.43 and 62.93% of LDH released from nonviable cells at 15.62, 31.25, 62.5, 125 and 250 µg/ml of *Aloe vera* and *Terminalia Chebula* herbal formulation. IC_{50} value of MCF-7 was found to be 250 µg/ml moreover, it was found that the spontaneous release of LDH from the target cells. This enzyme-release cytotoxicity assay is convenient reaction, including antibody-dependent and Tcell-mediated reactions. From this, it is clear that the Herbal formulation have effective anti- cancer potential against MCF-7 cell lines [31].

CONCLUSION

The study demonstrates the cytotoxic and anticancer potential of the *Aloe vera* and *Terminalia chebula* herbal

formulation based on the Siddha medicine system. Herbal formulations have been shown to be effective in treating cancer without causing harmful side effects and have the potential to treat concomitant infections in cancer patients. Further research is needed to develop a safe and effective drug for breast cancer based on this herbal formulation.

LITERATURE CITED

1. Ahuja R, Agrawal N, Mukerjee A. 2013. Evaluation of anticancer potential of *Terminalia chebula* fruits against Ehrlich Ascites Carcinoma induced cancer in mice. *Jr. Sci. Innov. Research* 2(3): 549-554.
2. Akev N, Candoken E, Kuruca SE. 2020. Comparative study on the anticancer drug potential of a lectin purified from aloe vera and aloe-emodin. *Asian Pacific Journal of Cancer Prevention* 21(1): 99.
3. Akev N, Turkay G, Can A, Gurel A, Yildiz F, Yardibi H, Uzun H. 2007. Tumour preventive effect of Aloe vera leaf pulp lectin (Aloctin I) on *Ehrlich ascites* tumours in mice. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives* 21(11): 1070-1075.
4. Alves DS, Pérez-Fons L, Estepa A, Micol V. 2004. Membrane-related effects underlying the biological activity of the anthraquinones emodin and barbaloin. *Biochemical Pharmacology* 68(3): 549-561.
5. Amjed S, Junaid K, Jafar J, Amjad T, Maqsood W, Mukhtar N, Ansari F. 2017. Detection of antibacterial activities of Miswak, Kalonji and *Aloe vera* against oral pathogens & anti-proliferative activity against cancer cell line. *BMC Complementary and Alternative Medicine* 17(1): 1-10.
6. Asha MA, Senthilkumar RS. 2020. Green synthesis and characterization of silver nanoparticles from *Ocimum basilicum* and their antimicrobial antioxidant and anticancer activity. *Research Journal of Pharmacy and Technology* 13(12): 5711-5715.
7. Azghani AO, Williams I, Holiday DB, Johnson AR. 1995. A beta-linked mannan inhibits adherence of *Pseudomonas aeruginosa* to human lung epithelial cells. *Glycobiology* 5(1): 39-44.
8. Basak P, Paul S, Majumder R. 2017. Invitro cytotoxic study of aloe vera whole leaf extract on PBMC and breast cancer cell line. Paper presented at the 2017 2nd International Conference for Convergence in Technology (I2CT).
9. Birla S, Tiwari V, Gade A, Ingle A, Yadav A, Rai M. 2009. Fabrication of silver nanoparticles by *Phoma glomerata* and its combined effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Letters in Applied Microbiology* 48(2): 173-179.
10. Boudreau MD, Beland FA. 2006. An evaluation of the biological and toxicological properties of *Aloe barbadensis* (miller), *Aloe vera*. *Journal of Environmental Science and Health Part C* 24(1): 103-154.
11. Bupesh G, Manikandan E, Thanigaiarul K, Magesh S, Senthilkumar V. 2016. Enhanced antibacterial, anticancer activity from *Terminalia chebula*. Medicinal plant rapid extract by phytosynthesis of silver nanoparticles core-shell structures. *Jr. Nanomed. Nanotechnology* 7: 355.
12. Byeon SW, Pelley RP, Ullrich SE, Waller TA, Bucana CD, Strickland FM. 1998. *Aloe barbadensis* extracts reduce the production of interleukin-10 after exposure to ultraviolet radiation. *Journal of Investigative Dermatology* 110(5): 811-817.
13. Carounanidy U, Satyanarayanan R, Velmurugan A. 2007. Use of an aqueous extract of *Terminalia chebula* as an anticaries agent: A clinical study. *Indian Journal of Dental Research* 18(4): 152.
14. Cheng B. 2016. Development of a chemiluminescent immunoassay for cancer antigen 15-3. *Labeled Immunoass. Clin. Med*. 23(11): 1348-1351.
15. Cheng HY, Lin TC, Yu KH, Yang CM, Lin CC. 2003. Antioxidant and free radical scavenging activities of *Terminalia chebula*. *Biological and Pharmaceutical Bulletin* 26(9): 1331-1335.
16. Chhabra S, Mishra T, Kumar Y, Thacker G, Kanojiya S, Chattopadhyay N, Trivedi AK. 2017. Chebulinic acid isolated from the fruits of *Terminalia chebula* specifically induces apoptosis in acute myeloid leukemia cells. *Phytotherapy Research* 31(12): 1849-1857.
17. Chithra P, Sajithlal G, Chandrakasan G. 1998. Influence of Aloe vera on collagen characteristics in healing dermal wounds in rats. *Molecular and Cellular Biochemistry* 181(1): 71-76.
18. Easom KA, Klabunde KJ, Sorensen CM, Hadjipanayis GC. 1994. Nanoscale magnetic particles. New methods to surface protected metallic and immiscible bimetallic clusters/particles. *Polyhedron* 13(8): 1197-1223.
19. El-Shemy H, Aboul-Soud M, Nassr-Allah A, Aboul-Enein K, Kabash A, Yagi A. 2010. Antitumor properties and modulation of antioxidant enzymes' activity by *Aloe vera* leaf active principles isolated via supercritical carbon dioxide extraction. *Current Medicinal Chemistry* 17(2): 129-138.
20. Gacche RN, Shaikh RU, Pund MM. 2011. In vitro evaluation of anticancer and antimicrobial activity of selected medicinal plants from Ayurveda. *Asian Jr. Trad. Medicine* 6(3) 1-7.
21. Gaidhani S, Lavekar G, Juvekar A, Sen S, Singh A, Kumari S. 2009. In-vitro anticancer activity of standard extracts used in ayurveda. *Pharmacognosy Magazine* 5(20): 425.
22. Gupta PC. 2012. Biological and pharmacological properties of *Terminalia chebula* Retz. (Haritaki)- An overview. *Int. Jr. Pharm. Pharm. Science* 4(3): 62-68.
23. Damak M, Gargouri Y, Bezzine S. 2011. In vitro study of the PLA2 inhibition and antioxidant activities of Aloe vera leaf skin extracts. *Lipids in Health and Disease* 10(1): 1-7.
24. Karole S, Shrivastava S, Thomas S, Soni B, Khan S, Dubey J, Jain DK. 2019. Polyherbal formulation concept for synergic action: A review. *Journal of Drug Delivery and Therapeutics* 9(1s): 453-466.
25. Kim HS, Kacew S, Lee BM. 1999. In vitro chemo preventive effects of plant polysaccharides (*Aloe barbadensis* Miller, Lentinus edodes, Ganoderma lucidum and Coriolus versicolor). *Carcinogenesis* 20(8): 1637-1640.
26. Kim HS, Lee BM. 1997. Inhibition of benzo [a] pyrene-DNA adduct formation by *Aloe barbadensis* Miller. *Carcinogenesis* 18(4): 771-776.
27. Murugesan MP, Ratnam MV, Mengitsu Y, Kandasamy K. 2021. Evaluation of anti-cancer activity of phytosomes formulated from aloe vera extract. *Materials Today: Proceedings* 42(2): 631-636.

28. Rai AK, Joshi R. 2009. Evaluation of antimicrobial properties of fruit extracts of *Terminalia chebula* against dental caries pathogens. *Jundishapur Journal of Microbiology* 2(3): 105-111
29. Reynolds T, Dweck A. 1999. Aloe vera leaf gel: a review update. *Journal of Ethnopharmacology* 68(1/3): 3-37.
30. Russell A, Hugo W. 1994. 7 antimicrobial activity and action of silver. *Progress in Medicinal Chemistry* 31: 351-370.
31. Russo A, Piovano M, Lombardo L, Vanella L, Cardile V, Garbarino J. 2006. Pannarin inhibits cell growth and induces cell death in human prostate carcinoma DU-145 cells. *Anti-Cancer Drugs* 17(10): 1163-1169.
32. Shalabi M, Khilo K, Zakaria MM, Elsebaei MG, Abdo W, Awadin W. 2015. Anticancer activity of *Aloe vera* and Calligonum comosum extracts separately on hepatocellular carcinoma cells. *Asian Pacific Journal of Tropical Biomedicine* 5(5): 375-381.
33. Shankara BR, Ramachandra Y, Rajan SS, Ganapathy PS, Yarla NS, Richard S, Dhananjaya BL. 2016. Evaluating the anticancer potential of ethanolic gall extract of Terminalia chebula (Gaertn.) Retz. (combretaceae). *Pharmacognosy Research* 8(3): 209.
34.Sharma GN, Dave R, Sanadya J, Sharma P, Sharma K. 2010. Various types and management of breast cancer: An overview. *Journal of Advanced Pharmaceutical Technology and Research* 1(2): 109.