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GENE EXPRESSION ANALYSIS OF *Aerva javanica* AND *Parkinsonia aculeata* SYNTHESIZED GOLD NANOPARTICLES IN BREAST AND COLON CANCER CELL LINES

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Synthesis of gold and silver nanoparticles from plants and its usages showing alternative treatment methods for various diseases. Cancer drugs conjugated nanoparticle drug delivery showing 50% more effective in cancer treatment compared to cancer drug alone. In our present study, Synthesized the AuNPs from *Aerva javanica* and *Parkinsonia aculeata* extract and studied the p53, caspase-3, Caspase-9 and NF-kB gene expression in MCF 7 and $CaCO_2$ cell lines by real-time PCR. Downregulation of gene expression was observed in *Aerva javanica* AuNPs and *Parkinsonia aculeata* AuNPs treated cells of MCF 7 and $CaCO_2$. Based on present results concluding the *Aerva javanica* AuNPs have more anticancer activity against to breast and colon cancer cell lines and further studies to be need in other cancers also.

Keywords: *Aerva javanica*; cancers; gene expression; gold nanoparticles; real-time PCR.

1. INTRODUCTION

All over the world medicinal plants are playing major role in the health [1]. Plant and extracts are playing an important role in the treatment of various human diseases including cancer [2]. In most of the developing countries, still 70-95% of the humans are using traditional medicines for many of the disease treatments [3]. The researchers are focusing on isolation of plant active principles and its uses due to its availability and less adverse effects. All over the world, Diabetes and cancers are leading death in human population. Day by day new cancer cases are increasing and 18.1 million cases are reported in year 2020 and 8.8 million in women and 9.3 million cases in men. There is need to search for the alternative drugs to treat the cancers with efficient activity and less toxicity.

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As per WHO, Cancer is the leading cause of death in worldwide and Breast cancer is the second leading cancer in women with 2.3 million womens are diagnosed with breast cancer globally in 2020.Many of the factors are cause to breast cancer like age, obesity, alcoholic and tobacco usage and 10 % of breast cancer cases are hereditary. As per National Cancer Registry Programme (NCRP) report, Breast cancers are a major health concern in Indian metropolitan cities compare to other cancers in females.

Colon cancer is a common lethal disease and reported the highest cases in Australia, New Zealand, Europe, and North America and Africa and South-Central Asia lowest incidents are reported [4]. As per the United States Surveillance, Epidemiology, and End Results (SEER) database under age 50 group colon cancer cases are increasing and decreasing in older groups [5,6]. The colorectal cancer incidences are lower in india compare to the western countries.

Aerva javanica (*A. javanica*) belongs to family Amaranthaceae and erect branched herb [7]. The whole plant contains sterols, phytols [8] and various parts of the plant also reported in anti-microbial, anti-ulcer, diuretic and nephroprotective activity [9]. A. javanica phytochemical analysis showed the polyphenols, terpenoids, flavonoids, and alkaloids are present [10].

Parkinsonia aculeata belonging to the fabaceae and grows up to 4-10m high and crooked trunk up to 40 cm diameter. Phytochemicals of this plant extracts showing anti-oxidant, anti-diabetic anti-bacterial and anti-malarial [11]. *In vitro* and *in vivo* studies of this plant contain flavonoids boost the metabolism and prevent the cancer through disposition of carcinogens [12,13]. In present work synthesized the gold nanoparticles and studied the gene expression in breast and colon cancers.

2. MATERIALS AND METHODS

2.1 Chemicals

Aerva javanica and *Parkinsonia aculeata* plants were collected and authenticated by Taxonomist. Gold (III)chloride hydrate (Cat No 254169-500MG) purchased from Sigma Aldrich. Dulbecco's modified Eagle's medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Trypsin EDTA, PBS were purchased from HiClone and Fetal Bovine Serum (FBS) were purchased from Gibco. Culture flasks and 6 well plated purchased from Corning.

2.2 Preparation of Plant Extracts

P 100 gm of dried plant powder soaked in 1000 ml water in conical flask for extraction and kept it for 72 hrs. with occasional shaking. After 72 hrs., the collected extracts were filtered and concentrated on rotary vapor using round bottom flask. Concentrated extract was stored at 4 ^{0} c used for further experiments.

2.3 Synthesis of Gold Nanoparticles (AuNPs)

125 µl of plant extract (60 g/100 ml H_2O) was mixed with 10 ml of 10mM gold chloride solution at room temperature. The color change Observed that the yellow-colored gold solution turned to wine red color within a minute. The synthesizedAu-NPs were characterized and stored at 4∘ C until further experiments.

2.4 Cell Lines and Culture Conditions

Breast cancer MCF-7 and Colon cancer $CaCO_2$ cell lines were procured from NCCS, Pune, India. and monolayered maintained in DMEM supplemented with 10% FBS and the antibiotics solution, in atmosphere of 5% CO_2 at 37^0C. Cell viability estimated by tryphan blue assay and cells were seeded in 6 well plate. After 24 hrs treatment of AuNPS, cells were scraped and isolated the total RNA using TRIzol reagent, Takara Bio Inc and isolated RNA is converted to cDNA using the Takara Bio single strand synthesis kit.

2.5 Gene Expression Analysis by Real-Time Polymerase Chain Reaction (RT-PCR)

Gene expression studied were studied by SYBER green using Qiagen Rotor Gene Q real time PCR. The PCR conditions were Initial denaturation 2 mins at 94^0C followed by 35 cycles denaturation 30 sec at 94^0C, annealing 60^0C and extension with 72^0C. Final extension will be 72^0C with 5 mins of reaction volume 20µl. All the reactions were run in triplicate, including no-template controls. The relative gene expression levels were calculated and tabulated.

2.6 Statistical Analysis

Data were processed using GraphPad Prism 7 software and the results were provided as a mean ± standard deviation (SD). The significance of a difference was considered in p-value < 0.05.

Table 1. Primer sequences of Genes

Gene Name	Primer Sequence
p 53	F AGAGTCTATAGGCCCACCCC R GCTCGACGCTAGGATCTGAC
Caspase-3	F AATTGCCTCCACACCTTCAC R TCACCAAGCTGCTCATCAAC
Caspase-9	F AAAGCCCCATCATTCTCCTT R CACCAGACTCGGCACAATC
NF-kB	F TAGCCACAGAGATGGAGGAG R CCGAGTCGCTATCAGAGGTA

3. RESULTS AND DISCUSSION

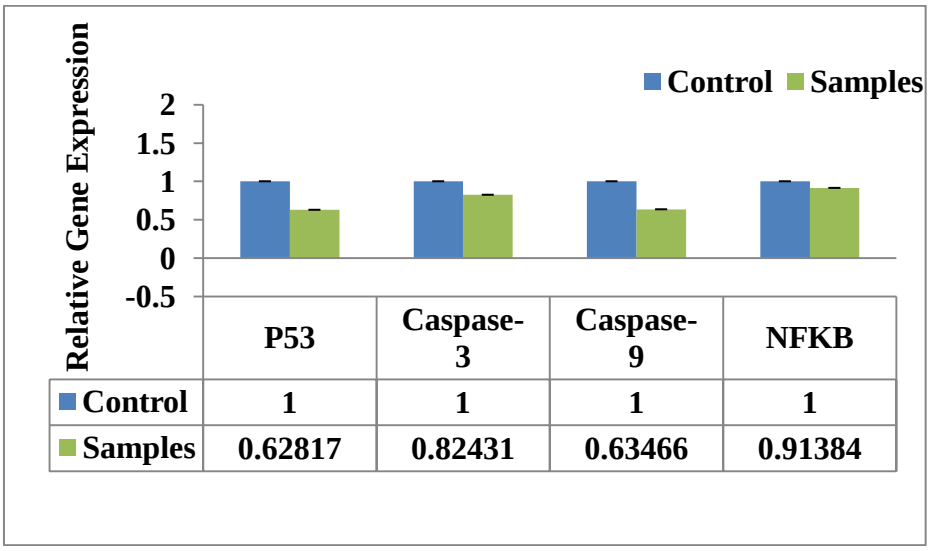
3.1 Results

Gold nanoparticles synthesized from *Aerva javanica* and *Parkinsonia aculeata* extracts (Fig. 1) treated with MCF-7 and $CaCO_2$ cell lines to study the p^{53}, caspase-3, caspase-9 and NF-KB gene expression by quantitative Real Time-PCR. In both cancers, Breast and Colon cancers studied genes were down regulated in gold nanoparticles treated cells compared to the untreated cells on MCF-7 and $CaCO_2$. Murraya koengii gold nanoparticles showed the prominent results in both cancers compares to the Punica granatum gold nano particles (Figs. 2 & 3).

A

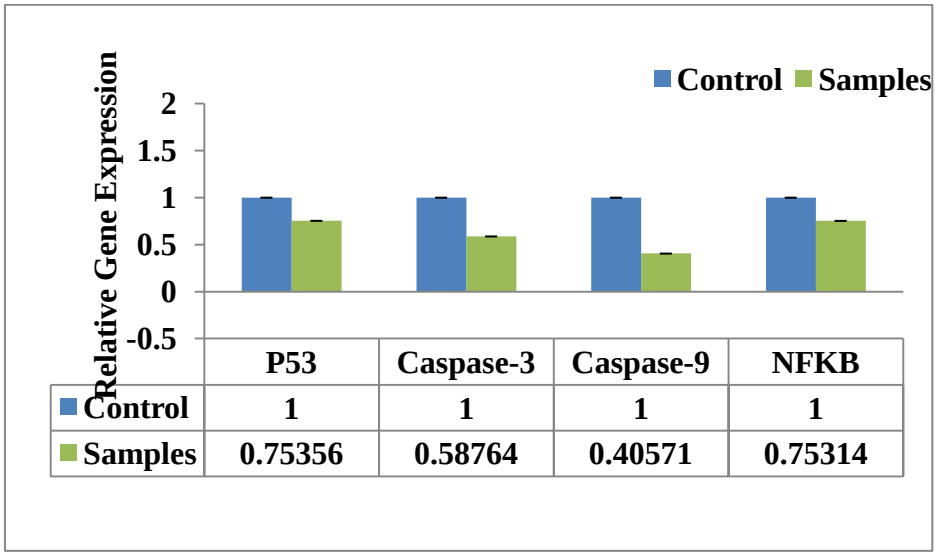
B

Fig. 1. Synthesis of Gold nanoparticles (A) *Aerva javanica* (B) *Parkinsonia aculeata*



	P53	Caspase-3	Caspase-9	NFKB
Control	1	1	1	1
Samples	0.62817	0.82431	0.63466	0.91384

A



	P53	Caspase-3	Caspase-9	NFKB
Control	1	1	1	1
Samples	0.75356	0.58764	0.40571	0.75314

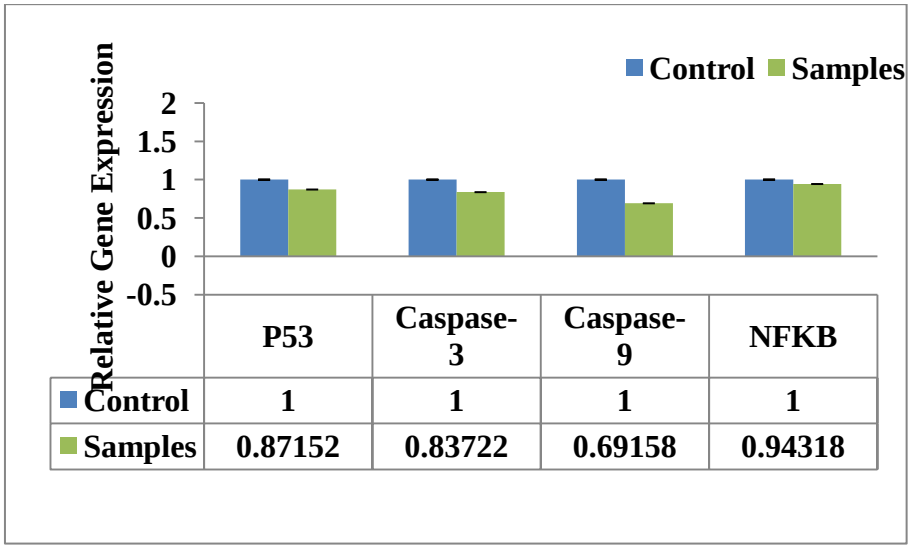
B

Fig. 2. Relative gene expression *Aerva javanica* Gold nanoparticles. (A) MCF-7 (B) $CaCO_2$cells. Results are expressed in Mean±SE (n=3)

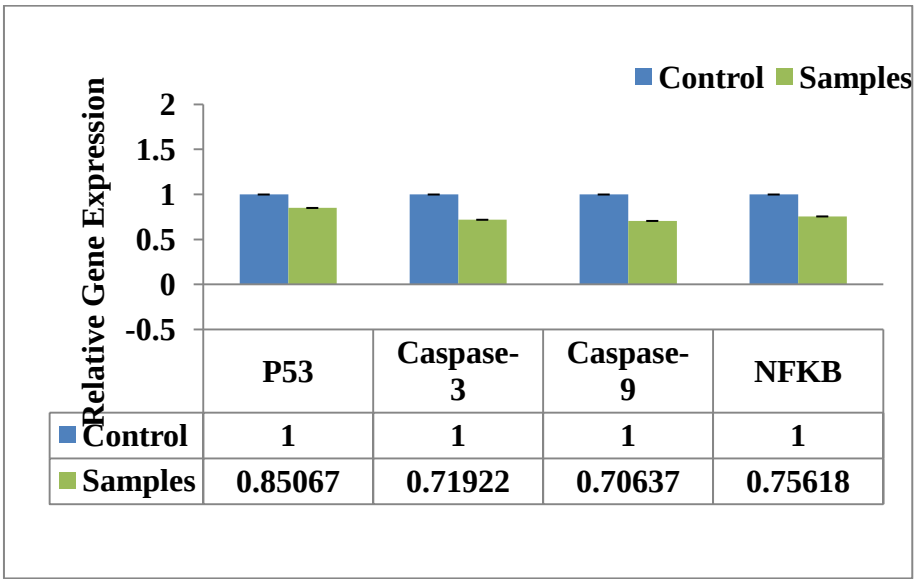
3.2 Discussion

Based on their unique properties of Gold nanoparticles (AuNPs) are widely using in bionanotechnology for different applications [14]. Nano particles synthesized from chemicals are showing toxicity due to generation of oxidative free radicals [15]. Synthesis of nanoparticles from plants are eco-friendly and less side toxic. Many years onwords chemotherapy is the effective treatment to cancers but biggest problem is multi drug resistance [16]. Standard cancer drugs combined with nanoparticles are delivered into tumor cells directly and showing more effective than standard drug alone [17] and this is the alternative promising delivery of anti-cancer drugs to tumor cells. In our present study synthesized the AuNPs from *Aerva javanica*, *Parkinsonia aculeata* plant extracts and studied the effect of this AuNPs on gene expressions in MCF 7 and $CaCO_2$cells by real time PCR. Das and Bavani studied the cytotoxicity of these two plants reported the *Aerva javanica* extract showed the better cancer activity compared to *Parkinsonia aculeata* extract [18]. Fozia Amin et al synthesized *Aerva javanica* leaf extract copper nanoparticles and studied the antimicrobial along with cytotoxicity and reported to

use the antimicrobial agents with negligible toxicity [19]. Mohammed Al-Shehri and Mahmoud Moustafa reported the extract of *A javanica* is a potent microbial agent for multidrug resistant microbes and promising candidate for treatment in breast cancers [20]. Nagla Mustafa Eltayeb et al reported the extracts of *Aerva javanica* showed the moderate anti-proliferative activity against breast cancer cell lines [21]. *Aerva javanica* extract synthesized AuNPs treated with MCF-7 cells showed the down regulation of P^{53}, Caspase-3, Caspase-9 and NF-KB follwos 0.62817, 0.82431, 0.63466 and 0.91384 folds. In $CaCO_2$ cells showed the down regulation of P^{53}, Caspase-3,Caspase-9and NF-KB follows 0.75356, 0.58764, 0.40571 and 0.75314 folds..Same way *Parkinsonia aculeata* extract synthesized AuNPs showed the downregulation of 0.87152, 0.83722, 0.69158 and 0.94318 folds in MCF7 Cells and 0.85067, 0.71922, 0.70637 and 0.75618 folds in $CaCO_2$ cells. Both AuNPs treated cells showed the down regulation in studied gene expressions compared to untreated control cells but *Aerva javanica* AuNPs showed more down regulation compared to *Parkinsonia aculeata* AuNPs in MCF 7 and $CaCO_2$ cell lines.



A



B

Fig. 3. Relative gene expression *Parkinsonia aculeata* Gold nanoparticles. (A) MCF-7 (B) $CaCO_2$cells. Results are expressed in Mean±SE (n=3)

4. CONCLUSION

Based on our present study results we are concluding the *Aerva javanica* AuNPs and *Parkinsonia aculeata* AuNPs treatment in MCF 7 and $CaCO_2$ cells showed the down regulation in gene expressions were observed and compared to *Parkinsonia aculeata* AuNPs and untreated control, *Aerva javanica* AuNPs showed more down regulation. Further studies to be need in other cancers also to conclude the same statement.

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 "traditional medicine" 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abdulrahman Ali Alzandi, Essam Ahmed Taher, Nageeb A Al-Sagheer, Abdul Wali Al-Khulaidi, Mohamed Azizi, Deyala M. Naguib.Phytochemical components, antioxidant and anticancer activity of 18 major medicinal plants in Albaha region, Saudi Arabia. Biocatalysis and Agricultural Biotechnology. 2021;34:102020.
2. Abhishek Bhanot**,** Rohini Sharma**,** Satnam Singh**,** Malleshappa N. Noolvi **,**Shashank Singh**.** *In vitro* Anti-Cancer Activity of Ethanol Extract Fractions of *Aerva lanata* L. Pakistan Journal of Biological Sciences. 2013;16(22):1612-1617.
3. Marcelo Fridlender, Yoram Kapulnik, Hinanit Koltai. Plant derived substances with anti-cancer activity: from folklore to practice. Front. Plant Sci; 01 October 2015.
4. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Allen C, et al. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. JAMA Oncol. 2017;3:524.
5. Brenner DR, Heer E, Sutherland RL, et al. National Trends in Colorectal Cancer Incidence Among Older and Younger Adults in Canada. JAMA Netw Open. 2019;2:e198090.
6. Abualkhair WH, Zhou M, Ahnen D, et al. Trends in Incidence of Early-Onset Colorectal Cancer in the United States Among Those Approaching Screening Age. JAMA Netw Open. 2020;3:e1920407.
7. Arbab AH, Parvez MK, Al-Dosari MS, AlRehaily AJ, Ibrahim KE, Alam P, Alsaid MS, Rafatullah S. Therapeutic efficacy of ethanolic extract of Aervajavanica aerial parts in the amelioration of CCl4-induced hepatotoxicity and oxidative damage in rats. Food & Nutririon Research. 2016;60:30864.
8. Movaliya V, Maitreyi Z. A Review on the Pashanbheda Plant Aervajavanica. International Journal of Pharmaceutical Science: Review & Research. 2014;25:268-75.
9. Mohammed Waqas, Peeyush Kumar Sharma, Mohammad Asif, Sandeep Yadav,Chandra Kala. Physicochemical, Phytochemical and Toxicological Study of Hydroalcoholic Extract of Aerva javanica Roots. Asian Plant Research Journal. 2021;8(2):35-40.
10. Chawla P, Chawla A, Vasudeva N, Sharma SK. A review of chemistry and biological activities of the genus Aerva a desert plant. Acta Pol Pharm. 2012;69:171-7.
11. Divya B, Mruthunjaya K, Manjula SN. Parkinsonia aculeata: A Phytochemical Review. Asinal Journal of Plant Sciences. 2011;10(3):175-181.
12. Priyam S, Rajni S, Manik S, Manvendra S. *In vivo* antitumor, antioxidant activities and toxicity profile of ethyl acetate crude leaf extract of Parkinsonia equality on B16F10 Melanoma. Int Res J Pharma. 2013;4:89.
13. Gupta M, Mazumder UK, Kumar RS, Sivakumar T, Vamsi ML. Antitumor activity and antioxidant status of Caesalpinia bonducella against ehrlich ascites carcinoma in Swiss albino mice. J Pharmacol Sci. 2004;94:177-84.
14. Yi-Cheun Yeh, Brian Creran, Vincent M. Rotello. Gold Nanoparticles: Preparation, Properties, and Applications in

Bionanotechnology. Nanoscale. 2012 Mar 21;4(6):1871–1880.
15. Martindale JL, Holbrook NJ. Cellular response to oxidative stress: signaling for suicide and survival. Journal of cellular physiology. 2002;192(1):1-15.
16. Abdel-Aziz,MS, et al. Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using Chenopodium murale leaf extract. Journal of Saudi Chemical Society. 2014;18(4):356-363.
17. Samra Khalid, Rumeza Hanif. Green biosynthesis of silver nanoparticles conjugated to gefitinib as delivery vehicle. International Journal of Advances in Science Engineering and Technology. 2017;5(2).
18. Pradip Das, M. Bavani Latha. Study The *In vitro* Anti-Cancer Activity of *Moringa oilfera*, *Aerva Javanica* and *Parkinsonia Aculeata* by Mtt Assay. Nat. Volatiles & Essent. Oils. 2021;8(4):14223-14233.
19. Fozia Amin, Fozia, Baharullah Khattak, Amal Alotaibi, Muhammad Qasim, Ijaz Ahmad, Riaz Ullah, et al. Green Synthesis of Copper Oxide Nanoparticles Using Aerva javanica Leaf Extract and Their Characterization and Investigation of *In Vitro* Antimicrobial Potential and Cytotoxic Activities. Evidence-Based Complementary and Alternative Medicine. 2021;Article ID 5589703:12.
20. Mohammed Al-Shehri, Mahmoud Moustafa Anticancer, Antibacterial, and Phytochemicals Derived From Extract of *Aerva javanica* (Burm.f.) Juss. ex Schult. Grown Naturally in Saudi Arabia. Tropical Conservation Science. 12:1–10.
21. Nagla Mustafa Eltayeb, Ghada Mustafa Eltayeb, Salizawati Muhamad Salhimi Anti-proliferative effect of *Aerva javanica* extracts on mcf7 and MDA-MB-231 breast cancer cell lines. Malaysian Journal of Analytical Sciences. 2017;21(5):1028–1035.