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# Study The In Vitro Anti-Cancer Activity Of Moringa Oilfera, Aerva Javanica And Parkinsonia Aculeata By Mtt Assay.

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**Abstract**

Cancer is anuncontrolled proliferation of malignant cells and it's a second leading cause of death in developed and developing countries. In Present study, evaluated the anticancer activity of Moringa oilfera, Aervajavanica, Parkinsonia aculeata plant extracts by MTT assay against the MCF7, HeLa, $CaCO_2$, $HepG_2$and A549 cell lines.Moringa olifera plant extract against MCF 7 shows the better anti-cancer activity with $IC_{50}$ of79.87±0.416 and least anti-cancer activity shows against of A549 with $IC_{50}$ of125.13±0.531µg/ml. Aerva javanicaplant extract against HeLashows the better anti-cancer activity with $IC_{50}$ of60.38±0.438and least anti- cancer activity shows against of $CaCO_2$ with $IC_{50}$ of95.65±0.582µg/ml.Parkinsonia aculeata plant extract against HeLa shows the better anti-cancer activity with $IC_{50}$ of 91.45 ±0.857 and least anti- cancer activity shows against of $CaCO_2$ with $IC_{50}$ of 178.04±0.476µg/ml. Based on present result concludes that Aerva javanica plant extract shows the better anti-cancer activity in studied cell lines compared to Moringa olifera and Parkinsonia aculeata plant extract.

**Key words:** Anti- cancer, MTT,Moringa oilfera, Aervajavanica, Parkinsonia aculeata

## 1. Introduction

All over the world, Cancer is one of the leading causes of morbidity and mortality in human population [1]. As per National Cancer Registry Programme Report 2020 by ICMR, India's cancer cases could increase by 12% in the next five years.In men, the new cancer incidences are estimated to be 763,575 in 2025 and in women, it is estimated to be 806,218 in 2025.In both men and women, cancers of the gastrointestinal tract are estimated to contribute 270,000 (19.7%) of the total cancer burden Breast cancer in women is estimated to contribute 200,000 (14.8%) and cervix cancer cases

about 75,000 (5.4%).In North America, Australia, New Zealand and Western Europe cancer incidence and mortality rates are higher compared to the rest of the world [2,3].In the United States, one in four deaths is attributed to cancer [4] . Behavioural and dietary risks such as physical inactivity, smoking, use of alcohol and having an unhealthy diet low in fruit and vegetables are one of the causatives for the cancers. Along with, Many other factors exposure to certain chemicals, metals and infectious agents also causative and progressive agents for the cancers [5].Surgery, Radiation and Chemotherapy are major ways to treat cancers. Chemotherapy has been using to treat cancers varying success but due to its side effects and resistance researchers are focusing on alternative to treat cancers and natural medicines extracted from plants are priority.

Medicinal plants are playing major role in the treatment of many diseases. The extract of these plants can act as anti-inflammatory, antioxidative, anti-allergic, anti-cancerous, analgesic, and antidiabetic. Due to these medicinal properties, these plants have been used since centuries for the cure and prevention of different kind of diseases. Derivatives from the medicinal plants and their extracts are effective in small amounts, economical, and safe to use, with negligible side effects. Moreover, medicinal plants are easily accessible and have better compatibility[6].

Moringa oleifera, commonly known as drumstick tree or horseradish tree and grows in the tropical and subtropical regions of the world and belonging to the family of Moringaceae [7]. Availability of different essential phytochemicals in leaves and seeds of Moringa olifera contains high nutritional values[8]. M. oleifera leaves have the high content of proteins has ideal levels of essential amino acids such as methionine, cysteine, tryptophan and lysine [9]. M. oleifera has a number of uses such as for human consumption and is a plant rich in antioxidant compounds which are of great importance in preventing tress causing several degenerative diseases[10]. A number of medicinal properties have been referred to various properties of this esteemed tree. The leaves, roots, barks, seeds and seed oils have been used for various ailments in South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal and haematological diseases[11].

Aerva javanica belonging to the family Amaranthaceae is a tall and woolly undershrub found plentiful in rainy season in Bhavnagar district of Gujarat state in India. Aerva javanica is reported as anthelmintic, diuretic, demulcent. It is used for the treatment of headache. The decoction of the plant is administered to remove swellings [12].In Ayurvedic medicines it is used as one of the best remedies for bladder and kidney stones and plant having anti-inflammatory anticalculus and insecticidal activity [13].

Parkinsonia aculeata belonging to the fabaceae is a small, spiny deciduous tree grows up to 4-10m high with short and often crooked trunk up to 40 cm diameter.Many pharmacological studied

reported the phytochemical constituents of this plant extracts shows the anti-bacterial, anti-malarial, anti-diabetic and anti-oxidant properties [14].

## 2.Materials and Methods

### 2.1 Collection and authentication Plant Material

Plant materials were collected and authenticated by Dr. Madhava Shetti, Taxonomist, Department of Botany, Tirupati. After collection of plant materials, washed thoroughly under running tap water until to the remove of adhering dust particles from the surface of the plants

### 2.2 Preparation of Plant extracts

Plant materials were shade dried and grinded to powder. and 10 gm of dried powder of plant material socked in 100 ml water in conical flask for extraction and kept it for 72 hrs. with occasional shaking. After 72 hrs., the extracts were filtered with No-42 whatman filter paper and collected water solvents concentrated on rotary vapor using round bottom flask. Concentrated extract was preserved in sterilized air tight labeled bottle and preserved in refrigerator at 4˚c until required for further use.

### 2.3 Anticancer activity

#### 2.3.1Materials and Methods:

DMEM (Dulbecco's modified Eagles medium), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS) and were purchased from Sigma Chemicals Co. (St. Louis, MO) and Fetal Bovine Serum (FBS) were purchased from Gibco. 25 $cm^2$ and 75 $cm^2$ flask and 96 well plated purchased from Eppendorf India.

#### 2.3.2 Maintenance of Cell Line:

The Human Cancer cell linesBreast cancer cell line (MCF-7),Cervical cancer cell line (HeLa),Colon cancer cell line ($CaCO_2$), Liver cancer cell line ($HepG_2$) and Lung cancer cell line (A549)were procured from NCCS, Pune and the cells were maintained in DMEM supplemented with 10 % FBS and the antibiotics penicillin/streptomycin (0.5 $mL^{-1}$), in atmosphere of 5% $CO_2$ /95% air at 37 $^0$C.

#### 2.3.3Preparation of Test Compound:

For MTT assay, Each Test compounds were weighed separately and dissolved in DMSO. With media make up the final concentration to 1 mg/ ml and the cells were treated with series of concentrations from 5 to 100 µg/ ml.

#### 2.3.4 Cell Proliferation Assay (MTT Assay):

Cell viability was evaluated by the MTT Assay with three independent experiments with six concentrations of compounds in triplicates. Cells were trypsinized and preform the tryphan blue assay to know viable cells in cell suspension. Cells were counted by hemocytometer and seededat density of 5.0 X $10^{3}$ cells / well in 100 µl media in 96 well plate culture medium and incubated overnight at 37 $^{0}$ C. After incubation, take off the old media and add fresh media 100 µl with different concentrations of plant extract in represented wells in 96 plates. After 48 hrs., Discard the drug solution and add the fresh medic with MTT solution (0.5 mg / $mL^{-1)}$ was added to each well and plates were incubated at 37 $^{0}$C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula.

$$\% \text{ Inhibition} = \frac{100\ (\text{Control} - \text{Treatment})}{\text{Control}}$$

The $IC_{50}$ value was determined by using linear regression equation i.e. y =mx+c. Here,

y = 50, m and c values were derived from the viability graph.

**2.3.5 Statistical Analysis**

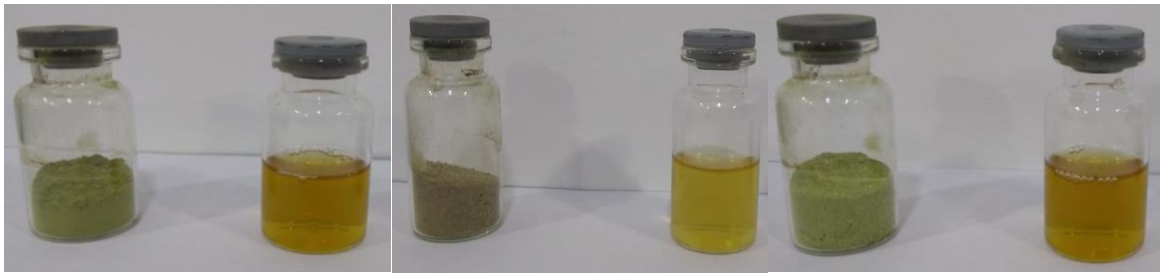
Data were processed using GraphPad Prism 7 software and the results were provided as a mean ± standard deviation (SD). The significance of a difference was considered in p-value < 0.05.

**3.Results and Discussion**

**3.1 Results**

The antiproliferative effects of three plant extracts with five cell lines determined by MTT method and cisplatin used as standard drug. For the MCF7, Moringa olifera plant extract shows the $IC_{50}$ of79.87±0.416 followed byAerva javanica80.93±0.382 andParkinsonia aculeata98.06±0.764µg/ml. Standard drug cisplatin shows the $IC_{50}$ of 5.62±0.141µM/mlrespectively**(Fig 2)**.For the HeLa, Aerva javanicaplant extract shows the $IC_{50}$ of 60.38±0.438 followed by Parkinsonia aculeata91.45±0.857andMoringa olifera95.37±0.278µg/ml.Standard drug cisplatin shows the $IC_{50}$ of 7.52±0.257µM/mlrespectively**(Fig 3)**. For the $CaCO_2$, Aerva javanicaplant extract shows the $IC_{50}$ of 95.65±0.582 followed by Moringa olifera 115.54±0.509andParkinsonia aculeata178.04±0.476µg/ml. Standard drug cisplatin shows the $IC_{50}$ of 116.47±0.842 µM/ml respectively**(Fig 4)**. For the $HepG_2$, Aerva javanicaplant extract shows the $IC_{50}$ of 85.63±0.572 followed by Parkinsonia aculeata104.33±0.285andMoringa olifera105.61±0.341µg/ml. Standard drug cisplatin shows the $IC_{50}$

of 11.72±0.183µM/ml respectively**(Fig 5)**.For the A549, Aerva javanicaplant extract shows the $IC_{50}$ of 77.50±0.914 followed by Moringa olifera125.13±0.531andParkinsonia aculeata127.95±0.872µg/ml. Standard drug cisplatin shows the $IC_{50}$ of 13.45±0.084µM/ml respectively**(Fig 6)**. The results are reported in **Table 1.**



**1A** **1B2A** **2B3A** **3B**

**Figure 1:**Plant Powder and Extracts. **1A)** Moringa olifera plant powder **1B)**Moringa olifera plant extract.**2A)** Aerva javanica plant powder **2B)** Aerva javanica plant extract.**3A)** Parkinsonia aculeata plant powder **3B)** Parkinsonia aculeata plant extract.

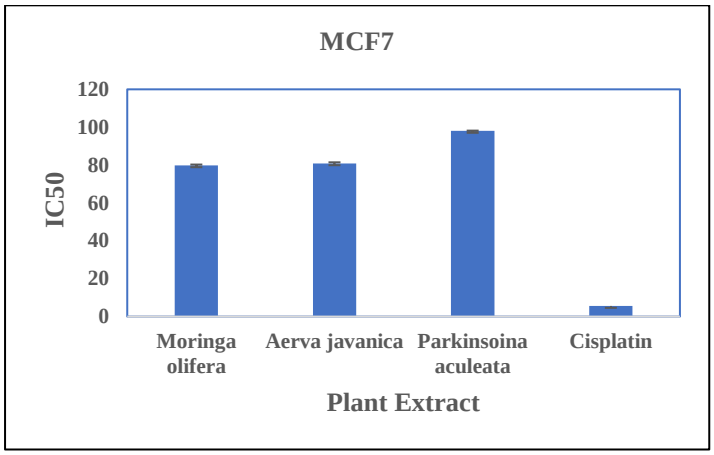



**Figure 2:** Cytotoxicity of MCF7 against Moringa olifera, Aerva javanica and Parkinsonia aculeata and standard Cisplatin.

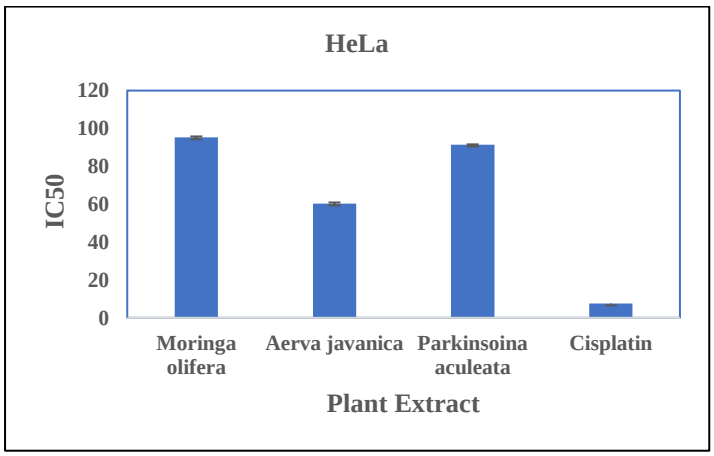


**Figure 3:**Cytotoxicity of HeLa against Moringa olifera, Aerva javanica and Parkinsonia aculeata and standard Cisplatin.

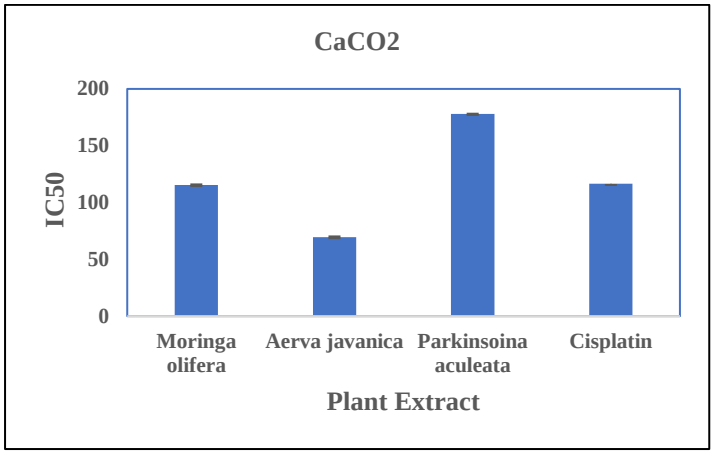



**Figure 4:** Cytotoxicity of $CaCO_2$ against Moringa olifera, Aerva javanica and Parkinsonia aculeata and standard Cisplatin.

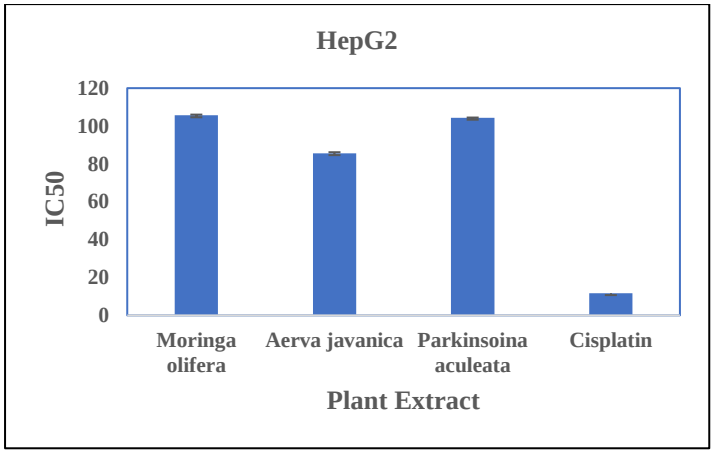



**Figure 5:** Cytotoxicity of $HepG_2$ against Moringa olifera, Aerva javanica and Parkinsonia aculeata and standard Cisplatin.

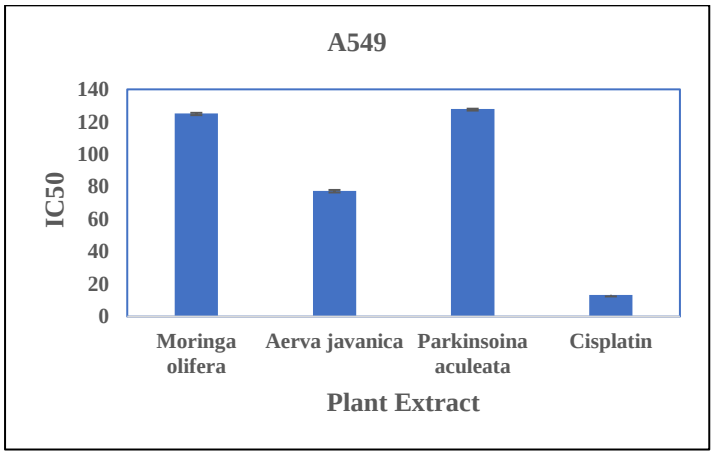


**Figure 6:** Cytotoxicity of A549 against Moringa olifera, Aerva javanica and Parkinsonia aculeata and standard Cisplatin.

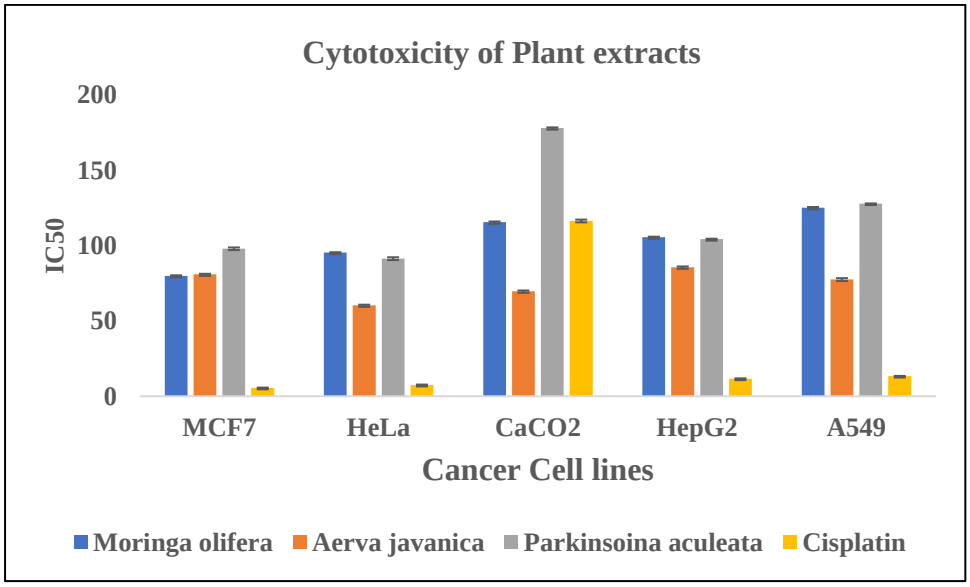



**Figure 6:** The cytotoxicity of the Plant Extracts is subjected to triplicates. The results are reported in mean±SD. The $IC_{50}$are reported in µg/ml. Standard drug cisplatin$IC_{50}$are reported in µM/ml.

| Plant Extract | MCF7 | HeLa | $CaCO_2$ | HepG2 | A549 |
|---|---|---|---|---|---|
| Moringa oleifera | 79.87±0.416 | 95.37±0.278 | 115.54±0.509 | 105.61±0.341 | 125.13±0.531 |
| Aerva javanica | 80.93±0.382 | 60.38±0.438 | 95.65±0.582 | 85.63±0.572 | 77.50±0.914 |
| Parkinsonia aculeata | 98.06±0.764 | 91.45±0.857 | 178.04±0.476 | 104.33±0.285 | 127.95±0.872 |
| Cisplatin | 5.62 ± 0.141 | 7.52±0.257 | 116.47±0.842 | 11.72±0.183 | 13.45±0.084 |

**Table 1:** The cytotoxicity of the Plant Extracts is subjected to triplicates. The results are reported in mean±SD. The $IC_{50}$are reported in µg/ml. Standard drug cisplatin$IC_{50}$are reported in µM/ml.

### 3.2 Discussion

Natural plants are playing a major role in disease treatment and prevention due to its pharmacological effects with minimum side effects with high biocompatibility [15].Moringa olifera is edible and rich sources of nutrients and using as a medicinal plant; vegetable; animal fodder; and a source of vegetable oil, which is used in condiments and the manufacture of perfumes, cosmetics, and hair care products[1617].Most of the medicinal properties of Moringa olifera like hypocholesterolemic, hepatoprotective, antimicrobial, anti-gastric ulcer,antiviral, and hypotensive are reported by many studies but onlyfew studies are reported use as anticancer drug [18].In our present research studied the anti-cancer activity of Moringa oliferaaqueous plant extract with five of

human cancer cell lines like MCF7, HeLa, $CaCO_2$, $HepG_2$ and A549 by MTT assay. This extract shows the good anti-cancer activity to breast cancer cells (MCF7) compared to the other studied cell lines. Kang Zi Khor et al revied the in vitro and in vivo anti-cancer property of moringa extracts and concluded the extract have huge potential to be developed in to anti-cancer drug and further research need to requires[19].Abdulrahman Khazim et al studied the anti-cancer effect of Moringa olifera plant parts against breast and colon cancer cell lines and suggested that both the leaf and bark extracts of Moringa collected from the Saudi Arabian region possess anti-cancer activity that can be used to develop new drugs for treatment of breast and colorectal cancers[20].Muhammad Zahid Mumtaz studied the Moringa olifera leaves extract with different solvents against HeLa cell line and reported the n-hexane fraction shows the 50 % inhibition with 416 µg/ml[21].

Aerva javanica has a many pharmacological properties like anti-bacterial and anti-cancer[22]. Ahmed H. Arbab studied the anti-oxidant and hepatoprotective activity of ethanolic aerva javanica extract and reported the potential of A. javanica in the attenuation of ex vivo and in vivo hepatotoxicity and oxidative damage [23].Desingu Kamalanathan and Devarajan Natarajanevaluated the anti-proliferative property of Aerva javanica plant extracts with MCF7 and shows the potent anticancer activity [24].In our present study the HeLa cells shows the better anti -cancer activity compared to the MCF7 and other cell lines.N. M. Eltayeb et al also studied the anti-proliferative effect of Aerva javanica solvent extracts on MCF7 and MDA-MB-231 breast cell lines and reported that moderate anti-proliferative activity against human breast cancer cell lines with some cytoselectivity towards MDA-MB-231 compare to MCF7 cells[25].

Parkinsonia aculeata is a well-known medicinal shrub for its beneficial effects as antipyretic, antimaterial, diaphoretic and abortifacient[26]. Butanol and hexane leaves extracts of Parkinsonia aculeata L. shows the potent antioxidant and free radical scavenging activity which may be due to the presence of flavonoids and polyphenols [27]. Along with this activity many of studied the toxicity of ethyl acetate crude extracts. This study proved the safety of dosage and ethylacetae leaf extract contain active principles are responsible for the anti-cancer property [28].SaharAbdelaziz et al studied the anti-oxidant and anti-cancer potentiality of Parkinsonia aculeata extracts. Results shows the ethyl acetate fraction shows the highest anti -oxidant activity and anti-cancer activity shows the MCF7 and HepG2 cell lines.

**3.3 Conclusion**

Anti-cancer activity of Moringa olifera,Aerva javanica and Parkinsonia aculeata studied with five types of cancer cell lines and based on the present study concluding that Aerva javanica plant extra shows the good anti-cancer activity against five cell lines compared to the

Moringa oliferaand Parkinsonia aculeata plant extracts with five cancer cell lines. Further studies to be needto carried out to know which active principle is responsible for the anti-cancer activity in aerva javanica.

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