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Antioxidant evaluation of herbal extraction and studies of their cytotoxic effect on human breast cancer cell lines (MCF-7 and MDA-MB-468)

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Abstract: Medicinal plant *Opuntia elatior* Mill., family *Cactaceae*, has been studied for its nutritional value and health benefit properties from fruit, where in it has been used for different disease conditions related to Asthma, Inflammatory disorder, hypoglycaemia, Anaemia, ulcer and diabetes. The fruit of the plants was extracted in sequential manner using petroleum ether, benzene, methanol and chloroform. Out of these, maximum extract yield present in the methanolic extract. Here also, it has been evaluated the antioxidant potential of petroleum ether, benzene, methanol and chloroform preparations of *Opuntia elatior* Mill Fruit material by Radical Scavenging by Hydrogen Radical Absorbance Capacity (DPPH), Ferrous Reducing Antioxidant Power (FRAP) Assay and their Cytotoxic Activity in Breast cancer cell lines,MCF-7 and MDA-MB-468. The petroleum ether (HP1), benzene(HP2), methanol(HP3) and chloroform(HP4) extracts of the fruit exhibited significant 'total antioxidant capacity' as determined by DPPH and FRAP method, however the HP3 showed higher Oxygen Radical absorbance Capacity than other. Methanolic (HP3) extracts showed significant cytotoxicity in breast cancer cell lines MCF-7 and MDA-MB-468, wherein HP3 showed cytotoxicity at much lower doses as compared to other. All these results suggest that *Opuntia elatior* Mill could be explored further for its anticancer potential with special reference to Breast cancer.

Keywords: *Opuntia elatior* Mill, antioxidant activity, Hydrogen Radical absorbance Capacity, Ferrous Reducing Antioxidant Power, cytotoxicity, Breast cancer, cell lines.

Introduction

Since last few years' science has focused on traditionally useful medically important plants and health benefits of foods and other related material. Nowadays medical professionals are

emphasizing on improvement of overall health and prevention of disease by using various plant extracts[36]. In this line, all types of wild fruits and vegetables have been recognized as valuable sources of neutraceuticals. The large number of natural product presents the chemically useful active compound and their multifunctional properties.*Opuntia elatior* on modification in glutamic oxaloacetic transaminase activity induce by *gamma* radiation in swiss albino Mice When mice are exposed to gamma radiation [1]. *Opuntia elatior*, a food item in some parts of the world, is known to have several neutraceuticals and pharmacologically active compounds [2-4-37]. The most important free radicals identified to induce oxidative damage cells are commonly termed as reactive oxygen species (ROS) and include superoxide anion (O_2^-), hydroxyl radical (OH^-), singlet oxygen (O_2^-), H_2O_2 and reactive nitrogen species (RNS) such as NO^-, NO_2^- and NO_3^- [5]. These free radicals cause membrane lipid peroxidation resulting into decrease in membrane fluidity, loss of enzyme receptor activity and damage to membrane proteins leading to cell inactivation [6]. The involvement of reactive oxygen species (ROS) in cancer progression has been demonstrated *in vitro* [7-9].

Antioxidants have been known to play an important role in alleviating the deleterious effects induced by free radicals by blocking the initiation or propagation of oxidizing chain reactions [10]. Synthetic and natural antioxidants have tremendous potency to prevent free radical formation, however, the former have often been found to be unsafe or toxic in the long term. Thus, the global interest has currently shifted towards the use of natural antioxidants, mostly present in herbs for health benefits [11]. According to the World Health Organization, around 80% of the world's population is resorting towards traditional medicines [12]. It has been reported that most of the cancer deaths could be prevented through appropriate dietary modification [13]. An inverse correlation between consumption of fruits, vegetables, spices, cereals and the risk of cancer disease suggest that antioxidants present in the plants act as effective agents for the cancer prevention [14]. Antioxidant rich diet may provide human health benefits to reduce the risk of cancer development. Dietary compounds are believed to function as anticancer agents through induction of cellular defence systems including the detoxifying and antioxidant enzymes as well as through inhibition of cell proliferation and inflammatory pathways leading to cell cycle arrest and/or apoptosis [15]. Antioxidants are the compounds that are less toxic, safe and are widely acceptable [16]. Polyphenols and flavonoids are the major antioxidant compounds present in different plants and have been shown to possess anticancer properties [17-18]. Moreover, antioxidants have also been used as adjuvants with anticancer drugs to achieve synergistic therapy [19].

Cactus (*Opuntia ficus-indica*), commonly known as prickly pear, belongs to the family *Cactaceae*. It is widely distributed in Mexico and in all American hemispheres as well as in Africa and in the Mediterranean basin [20]. It is well known that plants have been used in medicine for thousands of years. Particular relevance to this study, an extract of the Cactus plant, it has been used in traditional folk medicine because of its role in treating a number of diseases and conditions, including anti-inflammatory effects [21], hypoglycaemic effects [22], inhibition of stomach ulceration [23]. Cactus have been used in treating several diseases, such as rheumatic disease, hypertension, diabetes, asthma and gastric mucosa diseases traditionally use as medicine in many countries over the world. This plant contains bioactive molecules that are well known for their health-related properties [24]. Through antioxidant actions and also used for treating diabetes, burns, bronchial asthma and indigestion in many countries over the world [25] and effective in treating abdominal

cancer [26]. Recently, lot of work has been done to evaluate the antioxidant properties of fruits *Opuntia elatior* Mill (35)wherein antioxidant assay such as DPPH radical scavenging assay, reducing power assay (FRAP) have been studied.

In the present study, we have compared the 'total antioxidant capacity' of petroleum ether(HP1), benzene(HP2), methanol(HP3) and chloroform(HP4)extract of *Opuntia elatior* Mill fruits by DPPH and FRAP Assay. As well as cytotoxic potential of all the extracts in breast cancer cell lines. Our findings suggest that all the extracts exhibit significant antioxidant potential; however, the methanolic extract showed significantly higher total antioxidant capacity and cytotoxicity in Breast cancer cell lines viz.,MCF-7 and MDA-MB-468, thereby suggesting the chemo preventive potential of *Opuntia elatior* Mill in Breast cancer.

Materials and Method

All the chemicals used were of analytical grade.

Plant material and extraction

Collection and Authentication of plant:

The fruits of *Opuntia elatior* Mill were collected from road side weed near Billa Village, District Bhavnagar, Gujarat, India, authenticated by Dr. Bimal Desai (Assistant professor) Department of Botany ACHF, Navsari. These fruits were healthy and disease free and were used to check medicinal properties.

Preparation of fruit Extract:

Manually removed peel of fruits was subjected to air drying at ambient room temperature. Dry condition is required to prevent microbial contamination and subsequent degradation of metabolites. These fruits were kept away from direct sunlight to minimize chemical reaction which is caused by ultraviolet rays. After drying the fruits, they were grounded into a fine powder and passed through 10 mm sieve, which is then stored in an air tight container, in a dry and cool place. Grinding the fruits into a fine powder, for the extraction procedure, helps increase the surface area thus making it more homogeneous, and therefore making it easy for the solvent to penetrate the cells. A finely ground powder was extracted successively with petroleum ether (60-80 ^{0}C), benzene, chloroform, methanol in soxhlet extraction for 24 hours.

(a) (b)

Figure 1:(a) *Opuntia elatior* Mill. Plant. (b) *Opuntia elatior* Mill. Fruits.

Free radical scavenging activity by DPPH method

This method is simple and sensitive. The assay is based on the theory of hydrogen donor is an antioxidant. It measures compounds that are total radical scavengers. DPPH accept hydrogen from an antioxidant. The antioxidant effect is proportional to disappearance of DPPH in the test sample. These methods involve measurement of decrease in absorbance of DPPH at its absorption maxima of 516 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution [35].

The radical scavenging efficiency of petroleum ether (HP1), benzene(HP2), methanol(HP3) and chloroform(HP4)extracts of *Opuntia elatior* Mill. On the DPPH radical was estimated using the method of Brand-Williams *et al*. [27] with slight modifications. A freshly prepared methanolic DPPH solution (33 mg/L of methanol) was mixed with various concentrations of the extract (10, 20, 40, 80 and 160 µg/ml) in the ratio of 5:1 respectively. The contents were vigorously mixed, incubated at room temperature in the dark for 15minutes and the absorbance was measured at 517 nm using a UV-Spectrophotometer (Shimadzu). Methanolic DPPH solution along with solvent without extract served as a control. All the experiments were carried out in triplicates and repeated at least three times at different time points. The free radical scavenging capacity (RSC) of the tested compounds was expressed as percentage of DPPH elimination and was calculated according to the equation:

% RSC = absorbance of control - absorbance of extract) / absorbance of control ×100.

Reducing power assay

The standard spectrophotometric method [28] was used for the measurement of reducing power potential of petroleum ether(HP1), benzene(HP2), methanol(HP3) and chloroform(HP4)extracts of *Opuntia elatior* Mill Fruits. Various concentrations of both the extracts (10, 20, 40, 80 and 160 µg/ml) in 2.5 ml of phosphate buffer (pH 6.6) were mixed with 2.5 ml potassium ferricyanide (1%). The mixture was incubated at 50ºC for 15 min and around 2.5 ml TCA (10%) was added to it, followed by centrifugation at 3000 rpm for 10 min. A portion of the supernatant (2.5 ml) was taken to which 2.5 ml of water and 0.5 ml of $FeCl_3$ (0.1%) were added. Absorbance was measured spectrophotometrically at 700 nm and ascorbic acid was used as apositive control.

Cell lines &Cytotoxic assay

The human breast carcinoma cell lines, MCF-7 and MDA MB468 used in the study were obtained from National Centre for Cell Science (NCCS), Pune, India. The cells were grown in DMEM (Dulbecco's Modified Eagle Medium) containing 2mM L-glutamine supplemented with 10% foetal bovine serum and 100 U/ml of penicillin-streptomycin. The cells were incubated in a humidified 5-7 % CO_2 incubator at 37ºC.

The cytotoxicity of petroleum ether(HP1), benzene(HP2), methanol(HP3) and chloroform(HP4)extracts of *Opuntia elatior* Mill fruits was determined on breast cancer cell lines , MCF-7 and MDA MB468 and compared with a non-cancerous transformed cell line, VERO(Normal, Kidney) by MTT dye uptake [29]. The assay detects the reduction of MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide] by mitochondrial dehydrogenase to blue formosan product, which reflects the normal functioning of mitochondria and hence the cell viability. Briefly, MCF-7, MDA MB468 and ,VERO Cells were pre-incubated at a concentration of 1×10^6 cells/

mL in culture medium for 3 hrs at 37°C and 6.5 % CO_2, 75 % Relative Humidity. Cells were seeded at a concentration of 5 × 10^4 cells/well in 100 µl culture medium and various amounts of compound (final concentration e.g. 100 µm/ml-0.05 µm/ml) were added into micro plates (tissue culture grade, 96 wells, flat bottom). Cell cultures were incubated for 24 hrs at 37 °C and 6.5% CO_2. 10 µL MTT labelling mixture was added and incubated for 4 hrs at 37 °C and 6.5 % CO_2, 75 % Relative Humidity. 100 µL of solubilisation solution was added to each well and incubate for overnight. Absorbance of the samples was measured using a micro plate (ELISA) reader. The wavelength to measure absorbance of the formosan product is between 540 and 600 nm according to the filters available for the ELISA reader, used. (The reference wavelength should be more than 650 nm). After 24 hrs, the cytotoxicity data was evaluated by determining absorbance and calculating the correspondent chemical concentrations. Linear regression analysis with 95 % confidence limit and R^2 were used to define dose-response curves and to compute the concentration of chemical agents needed to reduce absorbance of the formazan by 50 % (IC_{50}).Percentage cell growth inhibition or percentage cytotoxicity was calculated by following formula:

% viability = $(A_T - A_B) / (A_C - A_B)$ × 100 … … … (1)

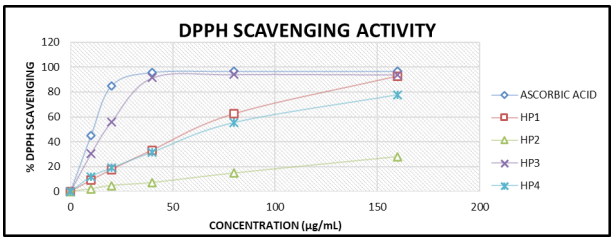
Where,

A_T = Absorbance of treated cells (drug)
A_B = Absorbance of blank (only media)
A_C = Absorbance of control (untreated)
There by,
cytotoxicity = 100 – % cell survival … … … (2)

Results and Discussion

Free radical concentration is greatly associated with disease and its progression. Therefore neutralization of Free radicals is of prime importance. To utilize herbal extract as drug, its antioxidant potential must be assessed as mentioned in methods.

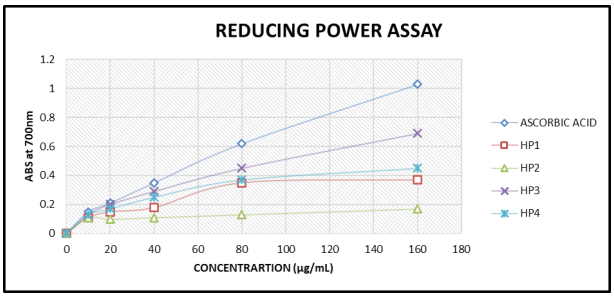
Plant extracts containing flavonoids and chlorogenic acid are highly effective in scavenging DPPH radical and in metal chelating capacity [30-31]. DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. As the concentration of Herbal extract increases, the % scavenging activity also increases. The HP3 extracts showed promising free radical scavenging effect of DPPH in a concentration dependent manner up to a concentration of 40 µg / mL after which the activity of extract got saturated. The scavenging activity of HP3 was greater than HP1, HP2 and HP4 (**Graph 1**).



Graph1: Free radical scavenging activity of petroleum ether(HP1), benzene(HP2), methanol(HP3) and chloroform(HP4) Extract of *Opuntia elatior*Mill fruits(10-160 µg/ml) were analysed for their free radical scavenging activity.

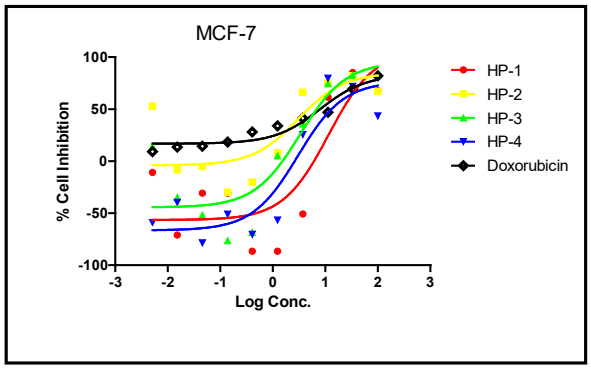
Present study also shows, all the extracts of three sources exhibited the reducing power in a concentration dependent manner. The reducing properties are generally associated with the presence of reductones. It has been reported that the antioxidant action of reductones was based on the breaking of the free radical chain by donating a hydrogen atom [32-33]. Reductones also react with certain precursors of peroxide, thus preventing

peroxide formation. Bioactive Compounds in extract act more or less similarly to reductones by donating the electrons and reacting with free radicals to convert them to a more stable product, and by terminating the free radical chain reaction. Results in the present study demonstrate that HP3 is more potent as compared to others **(Graph 2)**

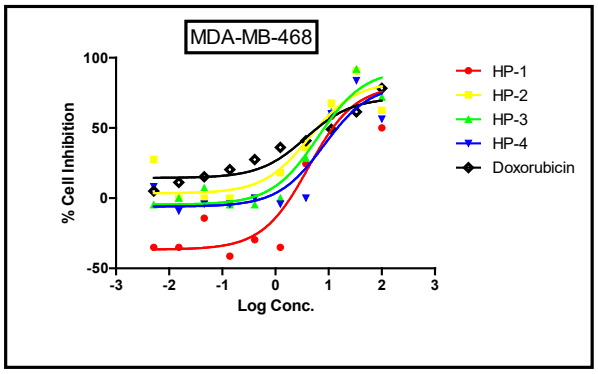


Graph 2: Reducing power activity of petroleum ether(HP1), benzene(HP2), methanol(HP3) and chloroform(HP4) extracts of *Opuntia elatior* Mill fruits(10-160 µg/ml) were analysed for their reducing power potential.

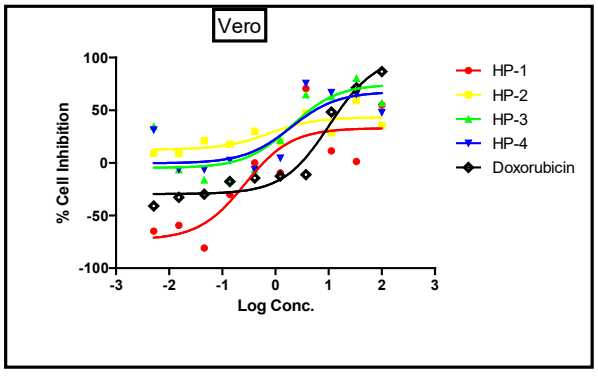
Cancer cell lines have been widely used as models of human cancer to better understand the biology of tumour formation and progression, as well as to help develop new therapeutic agents to treat the disease [34]. Due to side effect of conventional chemotherapy, nowadays it is essential to search a natural solution to cure/prevent breast cancer. With such aim we have tried various concentrations natural herbal extract on MCF-7 and MDA-MB-468 cell lines which represent breast cancer. The herbal extract HP3 showed good results as far as cytotoxicity is concerned. HP3 herbal seems to be very effective on MCF-7 and MDA-MB-468 cell lines than HP1, HP2 and HP4(Graph 3,4,5)



Graph 3. Sigmoidal curve-Dose response curve of extract of*Opuntia elatior* Mill fruits in various organic solventstested against MCF-7 cell line.



Graph 4.Sigmoidal curve-Dose response curve of extract of *opuntia elatior* Mill fruits in various organic solvents tested against MDA-MB-468 cell line.



Graph 5.Sigmoidal curve-Dose response curve of extract of *opuntia elatior* Mill fruits in

various organic solvents tested against Vero cell line.

Conclusion

The free radical scavenging activity of the extracts was evaluated by various biochemical assays. This assay provided useful information on the reactivity of the compounds with stable free radicals. In the presence study three herbal extract taken for the study. The fruit extract of HP3 possess significant antioxidant effect. Further research is in progress to identify the biomolecules responsible for the antioxidant activities.

Identification of antioxidant rich natural resources, preparing molecular fingerprints of their chemical compositions and studying the multiple therapeutic properties in this program may help make India self-reliant in drug development in future. It has been well known that plant has wide properties at molecular level that also affects protein expression and its function. To check out this we have prepared herbal extract especially from Cactus with various organic solvents *in vitro* on breast cancer cell lines (MCF-7 and MDA-MB-468) and the obtained results which are promising to use it as anti-breast cancer drug.

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