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ANTI-CANCEROUS AND ANTIOXIDANT POTENTIAL OF AQUEOUS EXTRACTS OF *ANNONA RETICULATA, PODOPHYLLUM PELTATUM, PSIDIUM GUAJAVA, ANANAS COMOSUS, CARISSA CARANDAS* ON MCF-7 CANCER CELL LINE.

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ABSTRACT

The aqueous leaf extracts of five medicinally important plants; *Annona reticulata, Podophyllum peltatum, Psidium guajava, Ananas comosus, Carissa carandas* were prepared to estimate their relative anti-cancerous efficiencies and their antioxidant potentials by estimating different antioxidant enzymes such as Catalase, Superoxide dismutase and Glutathione-S-Transferase and non-enzymatic antioxidant, Glutathione on MCF-7 cancer lines pretreated with different aqueous extracts . This study showed the antioxidant capacities of various extracts in the decreasing order as *Annona reticulata* > *Podophyllum peltatum* > *Carisa carandas* > *Annanas comosus* > *Psidium guajava*. *Annona reticulata* extract showed maximum protection of cell death in MCF-7 cell line as determined by cell viability assays and minimum cell death protection was exhibited by *Psidium guajava* extract. The present study reveals the great potential of these medicinal plants for future development of therapeutic drugs against breast cancer. These fruits can be a good source of natural antioxidants for both pharmaceutical and dietary requirements and appears to be useful in relieving oxidative stress.

KEY WORDS: Medicinal plants, anti-cancerous, MCF-7, cancer cell line, antioxidant enzymes, aqueous extracts

INTRODUCTION

Antioxidants neutralize free radicals, which are molecules with incomplete electron shells or the molecules that have lost an electron and thus are unstable. These are chemically more reactive than molecules with complete electron shells and basically steal electrons from other molecules in order to heal themselves, ultimately creating new free radicals in the process. By stealing electrons it can cause damage to DNA, leading to the possible development of cancer. Cigarette smoke, pollution, exposure to sunlight and several others- all cause the formation of free radicals. Other factors include normal daily processes like food digestion and breathing. They also pose to be useful in generation of ATP from ADP in the mitochondria during oxidative phosphorylation, apoptosis of effected or defective cells and likewise in many other ways.

Antioxidants are substances that prevent damage to cells caused by free radicals and search for free radicals, lend them electron which stabilizes the molecule, thus preventing damage to other cells. Antioxidants also turn free radicals into waste by products, and they eventually get eliminated from the body. They also have the ability to repair previous damage to cells. These antioxidants are found naturally in fruits and vegetables.

The plants taken are *Annona reticulate* (custard apple), *Podophyllum peltatum* (mayapple), *Psidium guajava* (guava), *Ananas comosus* (pineapple) and *Carissa carandas* (karonda) which have many medicinal properties and also show anti-cancerous effects.

Annona fruits are rich in calcium, phosphorous and vitamins B and C. They are believed to be powerful free radical scavengers. Guava contains major classes of antioxidant pigments giving it relatively high potential antioxidant value among plant foods. Extracts from guava leaves or bark are implicated in therapeutic mechanisms against cancer, bacterial

infections, inflammation and pain. Pineapple has been used to prevent ulcers, enhance fat excretion, helps in easy bowel movement, decrease severity of colds and infections, reduce swelling and bruising caused by surgery and injury, alleviate sinus inflammation, bronchitis, pneumonia; good for energy production and bone health; good for heart conditions. New research even shows pineapple to be highly effective at cancer prevention and treatment. Mayapple and its derivatives are used in several commercially available pharmaceutical products such as the anticancer drugs which are used in the treatment of small-cell lung cancer, lymphoblastic leukemia, testicular cancer and brain tumors. These are also used for psoriasis and malaria. The karonda fruit is a rich source of iron and contains a fair amount of vitamin C.it is antiscorbutic and very useful for cure of anaemia.

This paper aims at finding the most effective solution against the cancerous activities of free radical in terms of the naturally available plant extracts and is designed to estimate the relative anti-oxidative and anti-cancerous efficiencies of the most widely available but yet rarely used material- plant extracts. It covers from estimating the direct effects of the filtered plant extracts on cultured and well maintained cancerous cell lines (MCF-7) in terms of the effect caused to the cell count. This then is suitably backed by the relative data available from the measured anti-oxidative efficiencies of each of these extracts which include tests related to different primary antioxidants primarily concerned with anti-cancerous effects.

REVIEW OF LITERATURE

The events of World War II (1939-1945) led directly to the birth of free radical biochemistry. In 1954, Gershman and Gilbert speculated that the lethal effects of ionizing radiation might be ascribed to formation of reactive oxygen species (ROS). Since then free radicals (atoms with an unpaired electron) such as ROS and reactive nitrogen species (RNS) have gained notoriety (1) and there is also an increasing interest in the role of free radical - mediated damage in the etiology of human diseases. When the “attacked” molecule loses its electron, it becomes a free radical itself, beginning a chain reaction. Once the process is started, it can cascade, finally resulting in the disruption of a living cell. Free radicals have been implicated in the etiology of several human diseases as well as aging (2). In the status of normal metabolism, the levels of oxidants and antioxidants in humans are maintained in balance, which is important for sustaining optimal physiological conditions (3-4). Overproduction of oxidants in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA, and proteins (5). Oxidative damage to body cells and molecules has been widely postulated to be involved in the causation and progression of a range of chronic diseases (6). Cell damage caused by free radicals appears to be a major contributor to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction (7). Overall free radicals have been implicated in the pathogenesis of at least 50 diseases (8-9). Human metabolism counts on an antioxidant defensive system involving enzymes and proteins to prevent these effects. However, the defenses can be overwhelmed in certain circumstances so that harmful effects occur. It is accepted that the intake of antioxidant substances reinforces defenses against free radicals. The use of synthetic antioxidants has been limited because of their toxicity (10). Therefore, it is of great significant and necessity that research focuses on discovering potential natural, effective antioxidants to replace the synthetic ones. It is widely accepted that fruits and vegetables have many healthful properties (11). Consumption of fruits is beneficial to health and contributes to decrease of the mortality rate of cardiovascular and other diseases (12-14). This positive influence is attributed to some natural antioxidant phytonutrients (15-18). The majority of the antioxidant capacity of a fruit may be from polyphenols- flavonoids, tannins, along with vitamin A, B, C, and E and carotenoids (19). In view of huge importance of fruits as antioxidant sources, a comparison of their antioxidant property of commonly consumed fruits was investigated in order to evaluate their extent antioxidant capacity. Six *in vitro* antioxidant methods have been used to compare the activity utilizing the aqueous extracts of six different fruit [namely, *Annona reticulate* (custard apple), *Podophyllum peltatum* (mayapple), *Psidium guajava* (guava), *Ananas comosus* (pineapple) and *Carissa carandas* (karonda)] samples *viz.* SOD, catalase, GST, GSH and cytotoxicity test by MTT assay. These major antioxidant enzymes are directly involved in neutralization of ROS and RNS (20-27). SOD, the first line of defense against free radicals, catalyses the dismutation of superoxide anion radical into hydrogen peroxide by reduction. The oxidant formed (H_2O_2) is transformed into water and oxygen by catalase (CAT). Glutathione reductase, a favoprotein enzyme, generates GSH, with NADPH as a source of reducing power (19, 20-25).

MATERIALS AND METHODS

Preparation of Aqueous Extracts

Fresh leaves of each plant were taken as sample and dried in oven for three days with temperature set at 50°C. After drying for three days the leaves were ground to form a fine powder. A conical flask was taken and 1 gram of finely ground powder of leaves was added to it along with 50 ml distilled water. The flask was kept in shaking water bath for 30 minutes with temperature set at 30°C. The suspension was centrifuged at 10000 rpm for 15 minutes at 4°C. The supernatant was filtered using Whatman's filter paper no. 1 and used as the aqueous extract. (28)

Antioxidant Enzyme Assays

Superoxide dismutase (SOD) was estimated by Kakkar et al., 1984 method using phosphate buffer (0.17M, pH 8.3), nitroblue tetrazolium (1.2mM), phenazine methosulphate (28mM), NADH (150mM) and aqueous extracts of all plant samples. Absorbance was recorded at 560 nm after incubating at 30°C for 30 minutes.

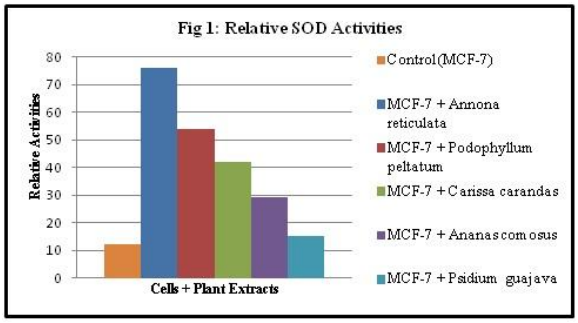
Catalase (CAT) was estimated by Ebby et al., 1974 method using phosphate buffer (50 mM, pH 7), hydrogen peroxide (10 mM), aqueous extracts of all plant samples and buffer substrate- H_2O_2 : Phosphate Buffer in ratio 10 µl : 100 ml. Readings were taken after every 2 minutes at an absorbance of 260 nm in decreasing order.

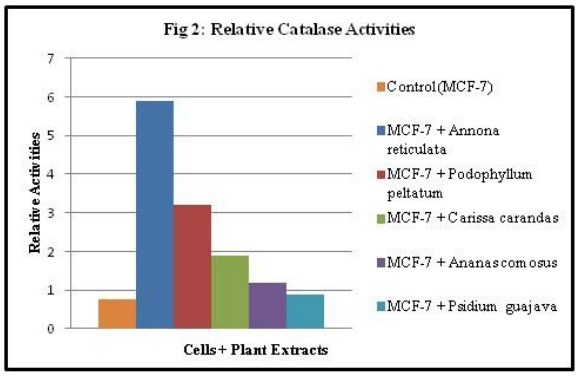
Glutathione-S-transferase (GST) was estimated by Habig et al., 1974 method using Phosphate Buffer (1M, pH 7), CDNB (1mM) solution in 5 ml ethanol, glutathione (1mM), PBS solution and aqueous extracts of all plant samples. Absorbance was recorded at 340 nm.

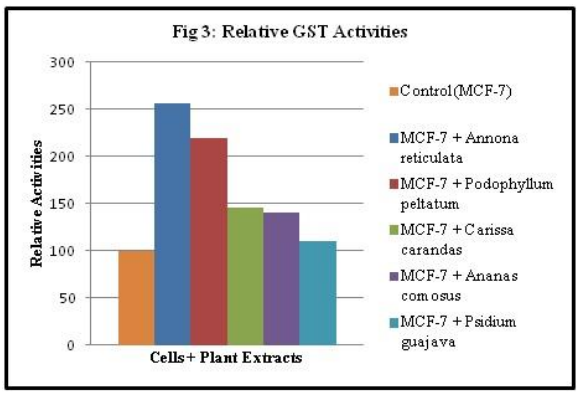
Reduced glutathione (GSH) was estimated by Moron et al., 1979 method using Phosphate buffer (1M, pH 7), GSH (0.001M), DTNB (Dithiobisnitrobenzoic Acid) (1mM) and aqueous extracts of all plant samples. Readings were taken at an absorbance of 412 nm.

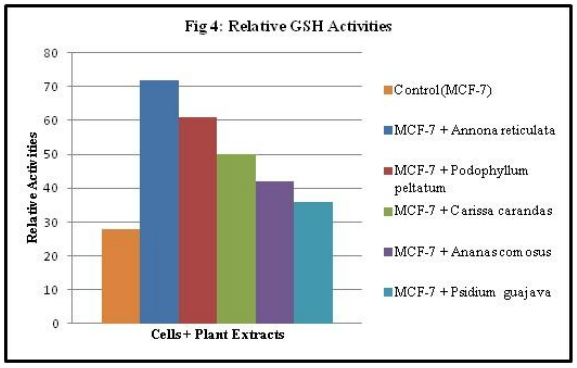
Cytotoxicity Assay was performed by Igarashi & Miyazava, 2001, using PBS (phosphate buffered saline), MTT (3mg/ml in PBS), isopropanol and aqueous extracts of all the plant samples. Samples were incubated for 3 hours at 37°C after adding MTT to them. After adding acid-propanol it was left in dark overnight. The absorbance was recorded at 650 nm.

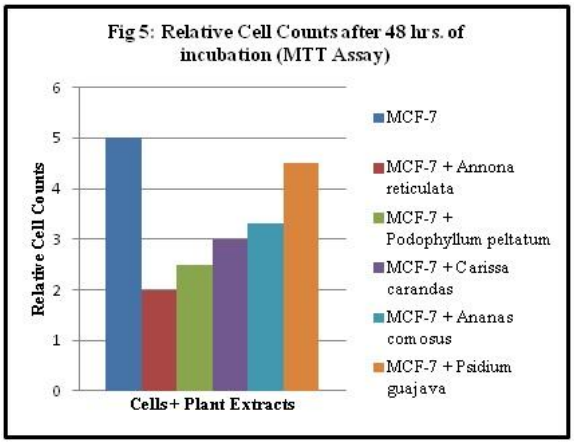
OBSERVATIONS











RESULTS AND DISCUSSION

The antioxidant potential of *Annona reticulata* was found to be the highest and that of *Psidium guajava* was the lowest.

Antioxidant Activity	*Annona reticulate*	*Podophyllum peltatum*	*Carissa carandas*	*Ananas comosus*	*Psidium guajava*
Catalase Activity (µM/min/ml)	5.9	3.2	1.9	1.2	0.9
SOD Activity (units/ml)	76	54	42	29	15
GST Activity (units/ml)	256	220	146	140	110
GSH Activity (µM/mg)	72	61	50	42	36
Cytotoxicity Assay (10^7 cells)	2	2.5	3.0	3.3	4.5

The catalase activity of *A. reticulata* was 5.9 µM/min/ml and that for *P. guajava* was 0.9 µM/min/ml. The SOD activity came out to be 76 U/ml for *A. reticulata* and 15 U/ml for *P. guajava*. The values for GST activity were 256 U/ml for *A. reticulata* and 110 U/ml for *P. guajava*. The GSH activity of *A. reticulata* was found to be 72 µM/mg and of *P. guajava* it was 36 µM/mg

The studies suggest that the leaf extracts of A. reticulata possesses potent in vitro antioxidant activity suggesting its role as an effective free radical scavenger, augmenting its therapeutic potential (*Bhaskar et al*., 2007).

Annona plant was evaluated with other plants for its anti-proliferative activity against HEp-2 (laryngeal cancer) and NCI-H292 (lung cancer) cell lines using the (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazole) (MTT) method, antioxidant capacity and tannin content . In addition, the antioxidant activity was evaluated with the DPPH (2,2-diphenyl-2-picrylhydrazyl) assay, and the tannin content was determined by the radial diffusion method. Plants with enhanced anti-proliferative activity (% living cells) were *Annona* plants, rich in tannins, cytotoxic compounds and antioxidant agents (*Gomes de Melo et al.,* 2010).

The ability of the aqueous extract of *Annona* leaves to scavenge free radicals in vitro and reversal of CCl_4-induced hepatocellular damage in rats suggest antioxidant and drug detoxification activities (*Ajboye et al.,* 2010).

The involvement of free radicals, especially their increased production, appears to be a feature of most, if not all human diseases, including cardiovascular disease and cancer (*Deighton et al.,* 2000). It has been found that cysteine, glutathione, ascorbic acid, tocopherol, flavonoids, tannins, and aromatic amines (phenylenediamine, p-aminophenol, etc.), reduce and decolourise DPPH by their hydrogen donating ability (*Blois,* 1958; *Yokozawa et al.,* 1998).

The present study proves that medicinal plants have great therapeutic potential and especially cancer, as proved above. Aqueous extracts of *Annona reticulata* show exceeding good results. Further study on the same can be of immense value for human welfare and this potential of medicinal plants can be used for further development of therapeutic drugs against breast cancer.

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