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Evaluation of Antioxidant Activity of Pomegranate Peels Phytosome

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Abstract: ***Phytosomes are novel phytophospholipid carriers for herbal drug delivery as standardized extracts which are shows, poor bioavailability and limited clinical utility. Pomegranate fruit (Punica granatum L.) belongs to the Punicaceae family is one of the most popular and important horticultural fruits, which contain high levels of a diverse range of photochemical including polyphenols, sugars, fatty acids (conjugated and non-conjugated), aromatic compounds, amino acids, tocopherol, sterols, terpenoids, alkaloids, etc. It has been proved that pomegranate has a high antioxidant activity and is effective in the prevention of atherosclerosis, cancer, cardiovascular disease, dental conditions, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage. The objective of this study to compare the antioxidant activity of methanolic extract of obtained from pomegranate peels and its Methanolic extract-phospholipid complex (ME-PC) phytosomes. Aqueous solutions of known Fe^{+2} concentrations, methanolic extract of pomegranate peels and prepared phospholipid complex were used for calibration. It was found that the methanolic extract- phospholipid complex i.e. prepared Phytosomes has higher antioxidant activity than methanolic extract.***

Keywords: Antioxidant, Pomegranate peels,Methanolic extract, Phytosome

1. Introduction

"Phytosomes" are novel phytophospholipid carriers for herbal drug delivery. The term **"Phyto"**means "Plant'', while "**some"** means "cell" like. Phytosomes are advanced microsphere or cell form of herbal product that are better absorbed, utilized to produce better result than those produced by conventional herbal extract. The water soluble phytoconstituent molecule can be converted in to lipid compatible molecular complexes which are called Phytosomes. Phytosomes produce little cell because of that valuable component of herbal extract are protected from destruction by digestive secretion & gut bacteria. Phytosomes are prepared by complexing the polyphenolicphyto constituent in the ratio of 1:2 or 1:1 with phosphotidylcholine.[1]Phosphotidylcholine is the principle building block of cell membranes adding fluidity and strength to cells[2]. It serves as a source of choline, an important nutrient for liver function and membrane a precursor of the neurotransmitter acetylcholine[3]and also promotes healthy liver and pancreatic antioxidant activity.[2]

Pomegranate fruit (*Punicagranatum* L.) belongs to the Punicaceae family is native to the region from northern India to Iran[4]. But it is also widely cultivated now in parts of South west America, California, Mexico, Arizona and Africa[5]Pharmacological effects of pomegranate represent a long history and have been mentioned in the Greek and Egyptian documents[6]. Recently, studies have shown that pomegranate has many potential effects including bactericidal[15], antifungal[15], antiviral, immune modulation, vermifuge, stimulant, refrigerant, astringent, stomachic, styptic, laxative, diuretic and an-thelmintic[7]. Moreover, it serves to decrease the adverse effects of cardiovascular diseases, diabetes, diarrhea, dysentery, asthma, bronchitis, cough, bleeding disorders, fever, inflammation[10], acquired immune deficiency syndrome, dyspepsia, ulcers, bruises, sores, mouth lesions, skin lesions, malaria, prostate cancer, atherosclerosis, hypertension, periodontal diseases, hyperlipidemia, male infertility, vaginitis, rectile dysfunction, Alzheimer, obesity and infant brain ischemia [4,7].Furthermore, pomegranate is an amazing source of cyaniding, delphinidin (both are anthocyanidins), caffeic acid, chlorogenic acid (both are phenolic acids), gallic acid, ellagic acid (tannic acids), luteolin, quercetin (flavones), Kaempferol (a flavonol), naringenin (a flavanone) as well as 17-alpha-estradiol, estrone, estriol, testosterone, beta-sitosterol, coumesterol, gamma-tocopherol, punicie acid, campesterol and stigmasterol in its juice, peels and seedoil that are chemopreventive and therapeutic potentials of this plant [10,11].It has been reported that the peel in particular possesses relatively higher antioxidant activity than seed and pulp [12].

Antioxidation is one of the most important mechanisms for preventing or delaying the onset of major degenerative diseases[7]. Active oxygen (hydroxyl, peroxy radicals, and single oxygen) is highly toxic and one of the strongest causative agents of many diseases, including cancer, heart disease, cataracts, and cognitive disorder. Antioxidants block the oxidation processes that contribute towards these chronic diseases and delay the onset of degenerative diseases of aging [13].

The body uses oxygen and nutrients to make energy. Oxygen also helps the immune system fight disease and harmful substances. Oxidation is a process that uses by products formed from oxygen fighting disease to create molecular agents that react with body tissues. Unfortunately, this process can form "free radicals" that cause cell damage. Antioxidants help reduce the number of free radicals that form in the body, lower the energy levels of existing free radicals, and stop oxidation chain reactions to lower the amount of damage caused by free radicals. The antioxidants of food are thought to prevent diseases caused by oxidative stress[14].

Free radicals are believed to be one of the causes of over sixty health problems, according to various scientific and medical groups. These problems include cancer, aging, and atherosclerosis. By increasing antioxidant intake and reducing exposure to free radicals. The enzymes are available in supplemental forms, but it is believed that taking the building blocks of the enzymes in supplemental form is more effective. Zinc, selenium, copper, and manganese are some of the building blocks. Minerals and vitamins are also often antioxidants. Vitamins including lutein,cysteine, beta-carotene, vitamin B2, vitamin C, vitamin E, and coenzyme Q10 and herbs[14]. Among the natural secondary metabolites, Phenolic compounds including flavonoids, Anthocyanin's and tannins, are the main group of antioxidant play a key role in the antioxidant mechanism by scavenging free radical produced during oxidation process.

Mechanism of action for antioxidant[13]

Two principle mechanisms of action have been proposed for antioxidants. first chain breaking mechanism by which the primary antioxidant donates an electron to the free radical present in system(lipid radical).The Second mechanism involves removal of ROS/RNS initiators (secondary antioxidants) by quenching chain initiating catalysts. Polyphenol compounds has ability to donate hydrogen atom and thus to act as chain breaking antioxidants, it can also chelate transition metal ions and hence inhibit free radical formation[13].

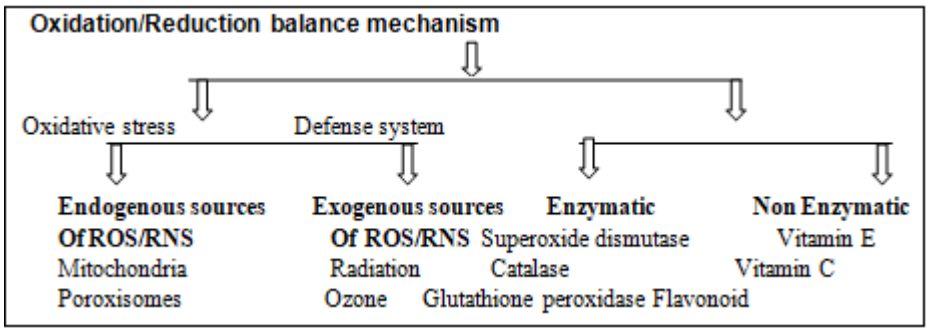

Figure 1: Oxidation/Reduction balance mechanism

2. Experimental

Material and Method

Collection & Authentication of plant Material

The Fruits were collected in the month of November to February from the surrounding areas of Aurangabad District, Maharashtra, India. The plant material was identified and authenticated by depositing the herbarium sheet of the plant specimens in Botany Department, Dr.BabasahebAmbedkarMarathawada University, Aurangabad, Maharashtra, India. Under the voucher No: Botany/2012-13/81.Accession no.0562.

Preparation of the Plant Extract[10,12]

Pomegranate fruits (500gm) were washed by distilled water then peeled and their edible portions were carefully separated. The peels were air dried in a ventilated oven at 40°C for 48 hours. Dried peels and coarsely powdered in an electric grinder and ground to a fine powder and passed through a 24 mesh sieve and stored in plastic bags for the next step. A 100gm sample of powder was subjected for extraction in Soxhlet extractor using petroleum ether, remove the steroidal content. The mark used for extraction of phenolic content using 200 ml methanol (99.9%) in soxhlet apparatus for 4-6 hrs. i.e.13-14 cycles.[10]Then extract were dissolved in 70% Methanol and Ethyl acetate in separating fennel shake for 30 min. separated the organic layer, excess of ethyl acetate was removed with help of rotary evaporator to produce a dry powder. and then subjected to preliminary chemical tests which revealed the presence of polyphenol component in the plant extracts. Polyphenolic compounds are determined by using HPLC.

Preparation of Phyto-Phospholipid complex by solvent evaporation method:[18]

Different molar concentration of Methanolic extract and Phosphotidylcholine (1:0.5, 1:1, 1:2) was taken in the beaker.20ml methanol was added in to the beaker. Placed beaker on magnetic stirrer for 2 hours .then kept into vacuum oven temperature below 60^0C till solvent was evaporated. Prepared phospholipids complex washed with 10 ml N- Hexane was added it with continuous stirring. The Flavonoid–phospholipid complex was precipitated and the precipitate was filtered and dried under vacuum to remove traces of solvents. The resultant phospholipids complex placed in an amber coloured bottle, flushed with nitrogen and stored at room temperature.

In vitro antioxidant study (Reducing power assay)[7,19]

The reducing power of methanolic extracts and Phytosome formulation was quantified by using 1 ml of reaction mixture, containing various concentrations of samples (0.05, 0.1, 0.2, 0.4, 0.8 and 1.2 mg/ml) in phosphate buffer (0.2 M, pH 6.6), was incubated with potassium Ferricyanide (1% w/v) at 50°C for 20 min. The reaction was terminated by adding trichloroacetic acid (TCA) solution (10% w/v) and the mixture was centrifuged at 2000 rpm for 10 min. The supernatant was mixed with distilled water and ferric chloride (0.1% w/v) solution and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

3. Result

The methanolic extract of pomegranate peels was found to contain high amount of Ellagic acid determined by High Performance Liquid Chromatography. In the present study methanolic extract-phospholipids complex was prepared to

improve the lipophilic properties of methanolic extract .The complex were prepared with different ratios of phospholipids and extract such as 0.5, 1, 2. The results showed that when the ratio was lower than 1, the stability of the phyto –phospholipids complex was worse. More stable phyto-phospholipids complex prepared with a 1:1 and 1:2 ratios of ingredients. Practical yield high was obtained when prepared with 1:2ratios. The obtained complex was used for the antioxidant assays. The result showed ME-PC has high antioxidant activity than simple methanolic extract.

4. Conclusion

Methanolic extract contain high amount of ellagic acid and ellagic acid is a potent antioxidant found in many plants and vegetables. In this protocol, we successfully prepared methanolic extract-phospholipids complex by solvent evaporation method. The obtained complex showed strong antioxidant activity may be due the presence of polyphenolic compounds.

5. Discussion

In present experiment we prepared methanolic extract of Pomegranate peels showed presence of Tannins, Flavonoids, and Phenolic compound. From HPLC study it was observed that extract contained high amount of Ellagic acid.

The lipophilic properties of Methanolic extract were improved by preparing complex with different ratios of phospholipids the results showed that when the ratio was lower than 1, the stability of the phyto –phospholipids complex was worse. The best phyto-phospholipid complex were prepared by using 1:1 and 1:2 ratios of ingredients.1.2 ratio has given high Practical yield of complex. The obtained complex was used for the antioxidant activity and result shows the complex was significantly higher than pure methanolic extract.

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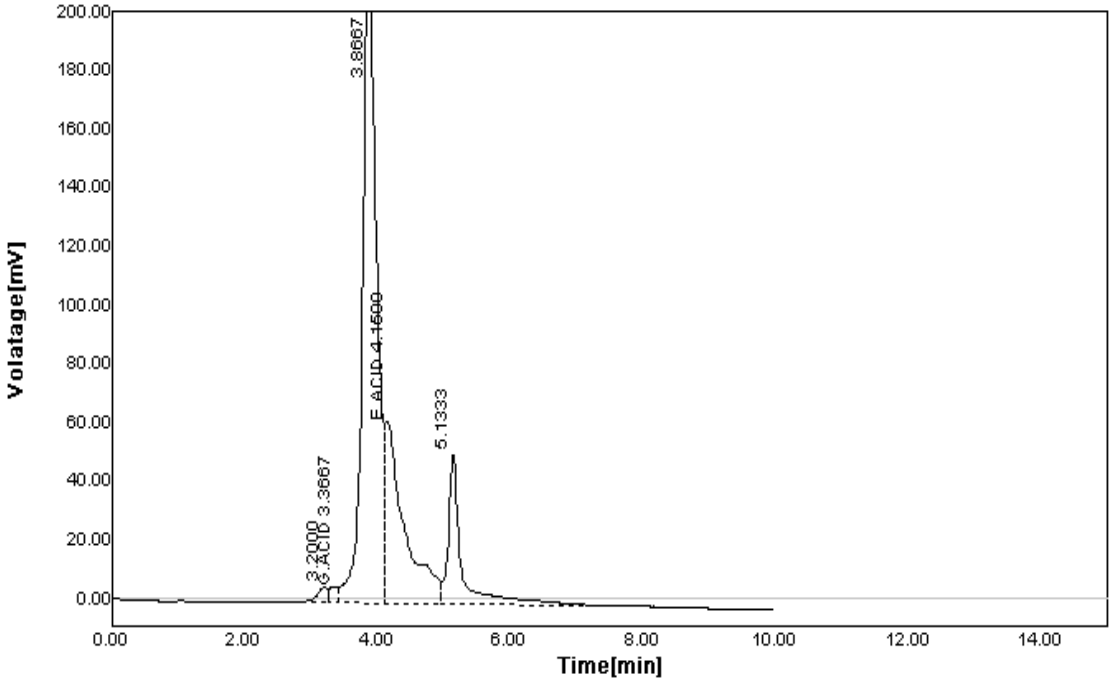

Figure 2: HPLC of Methanolic Extract of Pomegranate peels

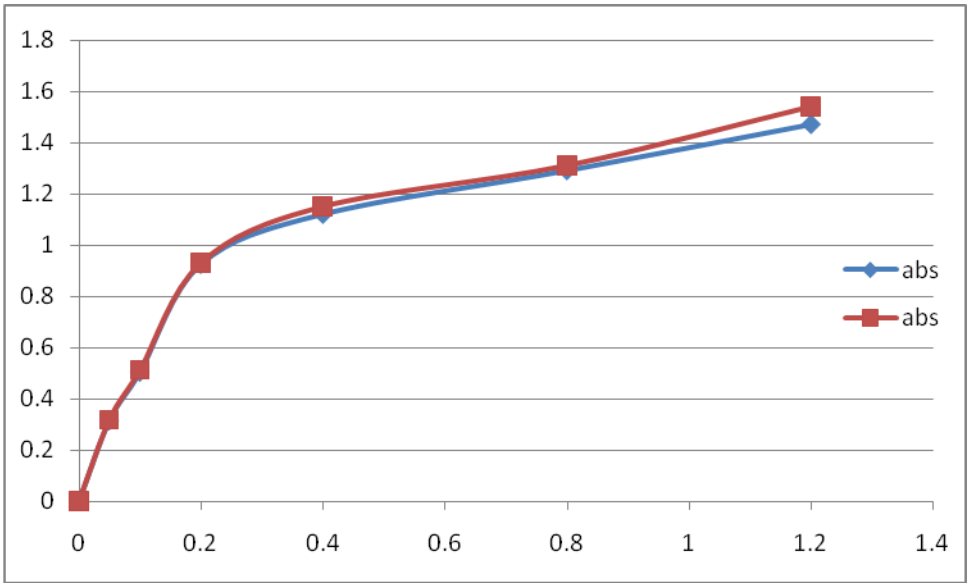

Figure 3: Reducing power of methanolic extract and ME-PC