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Original Article

Antioxidant and antitumor activities of Pomegranate (*Punica granatum*) peel extracts

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ABSTRACT

Antioxidant-rich fractions were extracted from pomegranate (*Punica granatum*) peels and seeds using ethyl acetate, methanol, methanol, hexan, chloroform and water. The extracts were screened for their potential as antioxidant using 1,1-diphenyl-2-picryl hydrazyl (DPPH) model systems. The methanol extract of peels showed 83 and 81% antioxidant activity at 50 ppm using the DPPH model systems. Similarly, the methanol extract of seeds showed 22.6 and 23.2% antioxidant activity at 100 ppm using the DPPH model systems. As the methanol, ethanol and water extract of pomegranate peel showed the highest antioxidant activity among all of the extracts; it was selected for testing of its effect on Ehrlich ascites carcinoma cells (EACC).

Keywords: *Punica granatum*; antioxidant activity; DPPH; Antitoumor activity

INTRODUCTION

Antioxidants are the compounds that when added to food products, especially to lipids and lipid-containing foods, can increase the shelf life by retarding the process of lipid per-oxidation, which is one of the major reasons for deterioration of food products during processing and storage. Synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytolune, have restricted use in foods as these synthetic antioxidants are suspected to be carcinogenic (Madhavi and Salunkhe, 1995). Therefore, the importance of the search for and exploitation of natural antioxidants, especially of plant origin, has greatly increased in recent years (Jayaprakasha, *et al.,* 2000). Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary healthcare system (Yineger and Yewhalaw 2007). For thousands of years, the practice of ayurvedic medicine has alleviated illness and attributed over all positive health (Samy *et al.,* 2008). Many plant-derived medicines used in traditional medicinal systems have been recorded in pharmacopoeia as agents used to treat infections and a number of these have been recently investigated for their efficacy against oral microbial pathogens (Devi *et al.,* 2011). Plants containing phytochemicals such as alkanoids, tannins, essential oils and flavanoids have pronounced defensive and curative activity. There are many species of medicinal plants belonging to various families which are being used, traditionally, to control and cure a variety of dental problems by the Indian population (Bhardwaj and Bhardwaj, 2012). The pomegranate (*Punica granatum* L.) is one of the oldest edible fruits and is widely grown in many tropical and subtropical countries (Salaheddin and Kader, 1984). Pomegranate juice and peel contain substantial amounts of polyphenols such as ellagic tannins, ellagic acid and gallic acid (Loren *et al.,* 2005). Pomegranate peel is a good source of antioxidants (Singh *et al.,* 2001). Extraction is a key step for obtaining antioxidants with an acceptable yield. Solvent extraction is more frequently used for the isolation of antioxidants and the extraction yield and economic viability is dependent on the type of solvent and method of extraction, mostly due to the differing polarity of these compounds. Several extraction techniques have been reported for the extraction of phenolic compounds from different matrices using solvents with different polarities, such as methanol, water, ethyl acetate and petroleum ether (Cheung *et al.*, 2003; Singh *et al.,* 2002). Furthermore, supercritical CO_2 (Palma *et al.,* 1999; Persson et al., 2002) and solvent extraction along sonication have been applied for this purpose (Bicchi *et al.,* 2000). The ripe Pomegranate fruit

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can be up to five inches wide with a deep red, leathery skin, is grenade-shaped, and crowned by the pointed calyx (Lansky and Newman, 2007; Kim *et al*., 2002). The aim of this research was to compare solvent extraction (acetone, methanol, ethanol, water and ethyl acetate) of *Punica granatum* L. as antioxidant and antitumor activity

MATERIALS AND METHODS

Collection of Fruits: The pomegranate was purchased from the local market. The seeds were separated from the rind and pith. The seeds were drained in a colander to remove any additional pith that is mixed with the seeds.

Preparation of Extracts: Pell extracts were prepared by cutting rind into small squares (approximately 5 mm^2) which was air dried, 5 gm of the dried material of rind was extracted with 200 ml methanol, ethanol, hexan and ethyl acetate using soxhlet apparatus for 6h. Extract is then is then distilled to remove solvent and used for testing antioxidant activity. Aqueous rind extract was prepared from 5 gm of the powdered rind is soaked in 100ml of distilled water for 48 hours and filtered. The filtrate is concentrated and used for testing antioxidant activity. Aqueous seed extract was prepared from 5 gm of the powdered seed is soaked in 100ml of distilled water for 24 h and filtered. The filtrate was concentrated. Aqueous pith extract was prepared from pith which was separated from the dry Pell and socked in 200 ml of distilled water overnight. It is then filtered and the filtrate concentrated and used for testing the antioxidant activity.

Phytochemical screening: The preliminary Phytochemical analysis was conducted for the presence of bioactive compounds such as alkaloids, glycosides, tannins, saponins, flavonoids, protein, carbohydrates and vitamin C as per the guidelines of Harbourne (1984), Raman (2006), Kokate *et al*. (1990) and expressed as negative (-) or positive (+).

Determination of total phenolics: The concentration of phenolic compounds in the extracts was determined according to the method of Jayaprakasha *et al.* (2001) and results were expressed as tannic acid equivalents. The pomegranate peel extracts were dissolved in a mixture of methanol and water (6:4 v/v). Samples (0.2 ml) were mixed with 1.0 ml of 10-fold-diluted Folin-Ciocalteu reagent and 0.8 ml of 7.5% Na_2CO_3 solution. After the mixture had been allowed to stand for 30 min at room temperature, the absorbance was measured at 765 nm using a UV–visible spectrophotometer (2401PC Shimadzu, Kyoto, Japan). The estimation of phenolic compounds in the fractions was carried out in triplicate, and the results were averaged.

Radical-scavenging activity of pomegranate peel extracts toward DPPH radical: The free radical-scavenging activity of pomegranate peel extracts were measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals using the method of Shimada *et al.* (1992). Five milliliters of DPPH ethanol soluteion (freshly prepared at a concentration of 0.1 mM) were added to 1 ml of pomegranate peel extracts. After 30 min, absorbance was measured at 517 nm using a UV–visible spectrophotometer (2401PC; Shimadzu, Kyoto, Japan). Ascorbic acid was used as a positive control. The experiment was carried out in triplicate and averaged.

Assay of the antitumor activity: The viability of tumor percentages of Ehrlich ascites carcinoma cells (EACC) which was measured by modified cytotoxic trepan blue exclusion technique (Bennett *et al.*, 1976).

RESULTS AND DISCUSSION

Preliminary Phytochemical analysis: The preliminary qualitative phytochemical analysis of the *Punica granatum* L. rind extract was carried out for detection of secondary metabolites and results were indicate that the Alkaloids and saponins was found to be absent showing the negative test in both solvents while glycosides tannins, vitamin C, carbohydrate, free amino acids and proteins was found to be present by the qualitative test. The **Table (1)** shows the presence and absence of secondary metabolite from the *Punica granatum* .The similar findings were also reported by Amina and Filali (2013), Hegde *at el.* (2012) and Satheesh Kumar (2012).

Biological activities: Free radical scavenging potentials of pomegranate peel extracts at different concentrations were tested by the DPPH method. Antioxidant reacts with DPPH, which is a stable free radical, and convert it to 1,1-diphenyl-2-picrylhydrazyl. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. The data obtained reveal that the extracts are free radical inhibitors and primary antioxidants that react with free radicals. In the DPPH assay, the radical scavenging capacity of polar extracts increased in a concentration dependent manner. The values for 50% scavenging activity (EC_{50}) are presented in **Figure (1).** The ethanol extract showed the highest radical scavenging activity (EC_{50}, 300 μg/mL), followed by water extract (EC_{50}, 350 μg/mL) and methanol extract (EC_{50}, 450 μg/mL).The polar extracts of *Punica granatum* were tested for their antitumor activity against

Ehrlich ascites carcinoma cells (EACC). The viability of EACC after incubation for 2h with each extract was determined as shown in **Figure (2).** The data showed that the viability of EACC was greatly increased by water extract followed by ethanol and methanol extracts. The antitumor activity of polar extracts may be due to the following reasons an interaction between extract compounds and proteins located on the membrane of cells. The activity of the extracts is attributed to their hydrogen donating ability (Shimada *et al.*, 1992). It is well-known that free radicals cause autoxidation of unsaturated lipids in food (Kaur and Perkins, 1991).

Table 1. Qualitative phytochemical analysis of *Punica granatum* (L) rind extract

Phytochemical	Test	MeOH	EtOH	Water	Hexan	CH_4	Ethyl Acetate
Alkaloids	Hager's	-	-	-	+	+	+
Glycosides	Keller Killiani	+	+	+	+	+	+
	Bromine water	+	+	+	+	+	+
Sapononin	Forth Foam	-	-	-	-	+	+
Tannins	Gelatin	+	+	+	+	+	+
Vitamin C	Dinitrophenyl hydrazine	+	+	+	+	-	-
Carbohydrate	Benedict's	+	+	+	-	-	-
Free Amino Acids	Ninhydrin	+	+	+	-	-	-
Proteins	Biuret	+	+	+	-	-	-

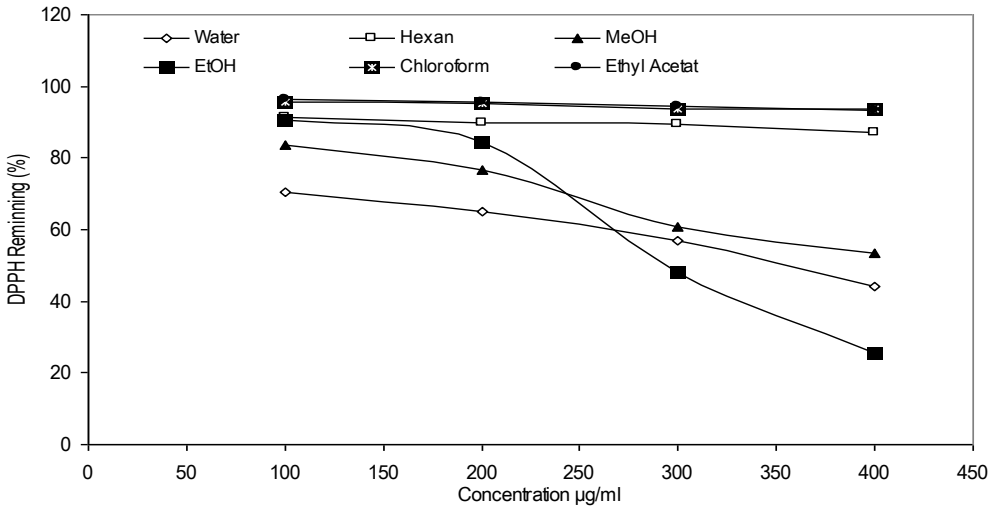

Figure (1) Antioxidant activity of different *Punica granatum* (L) rind extracts

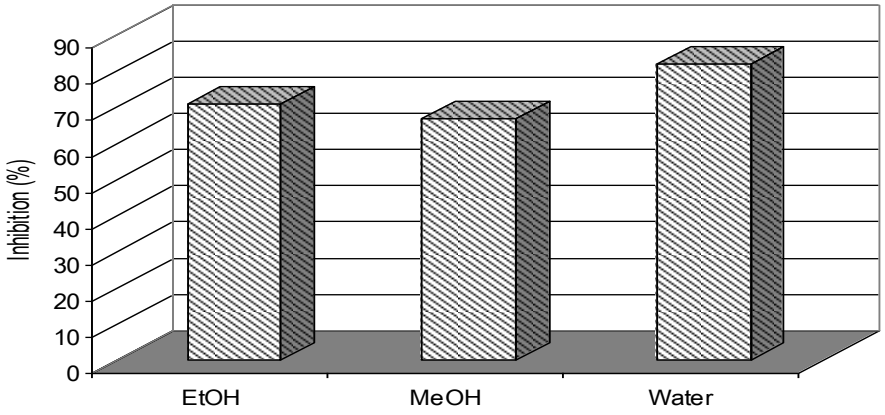

Figure (2) Anti-tumor activity of different *Punica granatum* (L) rind extracts against EACC

On the other hand, antioxidants are believed to intercept the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate further oxidation of the lipid (Sherwin, 1978). In biological systems, MDA is a very reactive species and takes part in cross-linking of DNA with proteins and also damaging the liver cells (Kubow, 1990). The free radical tends to be stabilized by a molecular rearrangement to produce a conjugated diene, which then easily reacts with an oxygen molecule to give a peroxy radical (Jadhav *et al.*, 1996). The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Gordon, 1990). This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. The ability of the pomegranate extract to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and seems to be a good scavenger of active oxygen species, thus reducing the rate of chain reaction. Oxidative modification is known to play an important role in the pathogenesis of atherosclerosis and coronary heart diseases (Steinberg *et al.*, 1989), and the dietary antioxidants that protect LDL from oxidation may therefore reduce atherogenesis and coronary heart diseases (Kinsella *et al.*, 1993). *P. granatum* has been extensively used as a traditional medicine in many countries for the treatment of dysentery, diarrhea, acidosis, hemorrhage and respiratory pathologies (Choi *et al.*, 2011). In addition, this plant is reported to have excellent antibacterial, antifungal, antioxidant and antitumor properties (Dahham *et al.*, 2010, Inabo and Fathuddin, 2011, Moussa *et al.*, 2011). Many phytochemical constituents have been reported to be present in different parts of the pomegranate plant making it pharmacologically precious (Prakash and Prakash, 2011).

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