ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Punica granatum (Pomegranate) Rind Extract as a Potent Substitute for L-Ascorbic Acid With respect To the Antioxidant Activity.

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ABSTRACT

L – Ascorbic acid finds its use in various food industries and cosmetic industries. It is most commonly known as Vitamin C and is popular because of its antioxidant activity. The antioxidant activity of L – Ascorbic acid is due to the presence of unique enediol grouping (-COH=COH-) which facilitates the scavenging of free radicals. Fruit peels or rind are basically the waste products of food and beverages industries. It is the outermost part of a fruit which is not preferred for consumption. *Pinus granatum* (commonly known as pomegranate), *Citrus sinensis* (commonly known as Orange) and *Citrus limetta* (commonly known as sweet lime) are few of the most common fruits being used in the juice industries and health product industries. In the current research, the rind of *Pinus granatum* has shown a better antioxidant activity than the L – Ascorbic acid, which is industrially used as an antioxidant agent in the food products. The Gas Chromatography Mass Spectrometer (GC-MS) study on the *Punica granatum* rind extract has shown the presence of various phenolic compounds, of which one is in abundance, namely, Phenol–2,4–Bis(1,1–Dimethylethyl)- and other cyclic compound named as Cyclotrisiloxane,Hexanemethyl-. These compounds with unsaturated bonds are supposed to have the capacity of free radical scavenging and hence the *Punica granatum* rind extract shows the potent antioxidant activity.

Keywords: Antioxidant, Ascorbic acid, Pomegranate, GC-MS, Vitamin C

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ISSN: 0975-8585

INTRODUCTION

Antioxidants are the free radical scavenging compounds which helps in prevention of various diseases due to free radical accumulation in the animal body. Ascorbic acid is one of the common antioxidants. Ascorbic acid has two enantiomeric forms, namely D – Ascorbic acid and L – Ascorbic acid [1]. The former is very rare in nature and can be chemically synthesized but the later is naturally found in various fruits and vegetables. L – Ascorbic acid, having the Molecular Weight of 176.12gm and the chemical formula as $C_6H_8O_6$, finds its use not only in various laboratory reagents but also in many of the consumable products ranging from food products, beverages to cosmetic products [2]. It is most popular by the name of Vitamin C and also known as Ascorbate. Naturally, it is available in various fruits and vegetables. Ascorbic acid is not only important for its industrial usage but also for many metabolic reactions inside the animal body. For example, it is required for collagen synthesis, neurotransmitter synthesis, tyrosine synthesis and many more metabolic reactions in the animal physiological system [3]. L-Ascorbic acid is more common than D-Ascorbic acid which is rarely found in natural system. In the food processing application, Ascorbic acid plays the double role by acting as a nutrient as well as an antioxidant agent at the same time. The most important chemical characteristic of Ascorbic acid is to have the unique enediol grouping (-COH=COH-) which facilitates the electron transfer reaction and hence imparts it the antioxidant activity [4]. Also, Ascorbic acid is being used in the cosmetic industry as it protects and strengthens skin cells and tissues against the oxidation, bacterial infection and formation of dead skin cells. It prevents the formation of wrinkles, keeps the skin rejuvenated, prevents the skin from losing its elasticity, and has various other cosmetic properties along with antimicrobial properties [5-7].

Punica granatum is a fruit bearing deciduous shrub, commonly known as Pomegranate. Since ancient Ayurvedic times, in the Indian medicines, the rind or the peel of the pomegranate fruit and the bark of the pomegranate tree has been known for several of its medicinal purposes. *Citrus sinensis* and *Citrus limetta,* commonly known as Orange fruit and Sweet lime fruit, are mostly used as fruit juices and several beverages products. The rind is the waste product of the food and beverage industries. The industry has to spend its capital in the disposal of the rind to prevent the prospective environmental pollution. If the rind can be used as a raw material for the manufacturing of a potent antioxidant compound then, it can have various advantages, such as eliminating the capital load of the industry in the disposal of the rind, adding up in the revenue generation by selling the rind and ultimately reducing the environmental pollution due to the decomposition of the rind.

In the current research study, the *Punica granatum* rind extract along with the rind extracts of *Citrus sinensis* and *Citrus limetta* have been studied for its antioxidant activity against the L – ascorbic acid as a standard antioxidant compound. The Reducing Power Assay for the antioxidant activity has been performed in the current study. The Reducing Power Assay revealed a very important property of the *Punica granatum* rind extract to be a better free radical scavenging agent than the L – ascorbic acid. Based on this fact, further GC – MS analysis was performed for the *Pinus granatum* rind extract. From the Gas chromatogram, the result obtained showed the presence of various phenolic compounds, of which two compounds are found to be abundant in the *Pinus granatum* rind extracts, namely Phenol-2,4-Bis(1,1-Dimethylethyl)- and Cyclotrisiloxane,Hexamethyl. These phenolic

compounds are supposed to be responsible for the potent antioxidant activity of the *Pinus granatum* rind extracts against the L-Ascorbic acid.

MATERIALS AND METHODS

Sample preparation

The fruit samples were collected from the Vellore Institute of Technology, Vellore campus, shopping complex. The rind and the fruit were separated manually. The rind was further washed with distilled water thrice to eliminate any foreign particles from the surface. 10% (w/v) (1gm of rind mixed with 9ml of distilled water) of the rind extract was prepared using distilled water. The mixture was ground properly using mortar and pastel to form a homogenized mixture. The homogenized mixture was sonicated to break the cell walls and extract the intracellular constituents of the rind solution. The sonication was done at amplitude 100, and pulsar 5 for 20 mins in case of each rind sample. The mixture after sonication was centrifuged at 3000rpm for 30 minutes to eliminate the solid particles which would become hindrance in the optical density measurement at 700 nm. The supernatant was collected for the Reducing Power Assay of the antioxidant activity.

Antioxidant assay

The Reducing Power Assay [8] has been used in the current study for determining the antioxidant activity of the sample. L-Ascorbic acid has been used as the standard or positive control for the current research. The Figure 1 explains the Reducing Power Assay. The Assay is based on the principle of conversion of Fe^{3+} ions to Fe^{2+} ions upon the action of the sample under study. This chemical conversion gives rise to a Yellow to Bluish color range, depending on the strength of the conversion. The pH of the phosphate buffer is maintained at 3.3 or in acidic range to facilitate the conversion of Fe^{3+} to Fe^{2+}, which takes places at acidic pH. The absorbance maxima of the color developed is at 700nm and is directly proportional to the antioxidant activity of the sample under study [9].

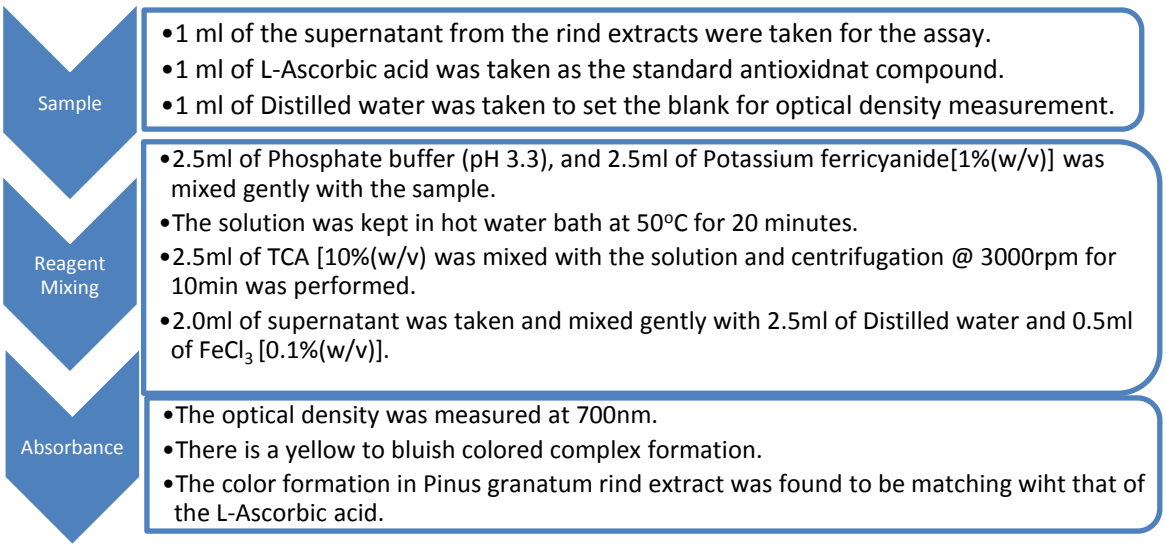

Figure 1: Schematic representation of Reducing Power Assay.

ISSN: 0975-8585

GC – MS analysis

The methanol extract of the rind was used for GC-MS sample. 0.5% (w/v) of the rind sample was prepared in methanol solvent. The sample was properly ground in a mortar pastel to obtain a homogenized mixture. The homogenized mixture was further sonicated for 100 minutes at amplitude 100 and pulsar 6. The mixture was poured in a sterile Petri dish and kept overnight to obtain a solid powdery sample of the rind. The powder sample was used for the GC-MS analysis. The GC-MS used in the present study was Perkin Elmer made with GC model as clarus 680 and Mass Spectrometer model as clarus 680(EI). The instrumental acquisition parameter was as following. Oven: initial temperature 60^{o}C for 2 minutes, ramp 10^{o}C/min to 300^{o}C, hold6 minutes, total run time = 32.0 minutes. Carrier gas was Helium and the column was Elite 5MS. The mass condition (EI) was kept as follows. Solvent delay = 2.00 minutes, Transfer temperature = 230^{o}C, Source temperature = 230^{o}C, Scan = 50 to 600 Da.

RESULTS

The *Pinus granatum* rind extract have shown a better antioxidant activity than L – Ascorbic acid as well as the other fruit rind extracts under the current scientific investigation on the basis of Reducing Power Assay being performed for evaluating the antioxidant activity. The Reducing Power Assay is a colorimetric assay and it uses the wavelength of 700nm to identify a characteristic compound formed by the reduction of Fe^{3+} to Fe^{2+}. From the Figure 2, it can be easily observed that the color developed for the *Pinus granatum* extract mixed with Reducing Power Assay reagents is similar to the color development in the standard solution which is L-Ascorbic acid in the current study. Further, the result of the optical density measurement at 700 nm as shown in Figure 3 proves the fact that *Pinus granatum* rind extract have more absorbance value than the L-Ascorbic acid and other fruit rinds in consideration. Hence, it is being inferred that Pinus granatum rind extracts are better antioxidative agent than the L-Ascorbic acid, based on the fact the absorbance value in the Reducing Power Assay is directly proportional to the antioxidant activity of the sample under consideration.

Table 1: Molecules identified in the *Pinus granatum* rind extract by GC-MS Analysis

Chemical compounds found in Pinus granatum rind extract	*Molecular Weight*	*Chemical formulae*
Phenol,2,4-Bis(1,1-Dimethylethyl)-	206	$C_{14}H_{22}O$
Phenol,3,5-Bis(1,1-diemthylethyl)-	206	$C_{14}H_{22}O$
3,4-diemthyl-2-(3-Methyl-Butyryl)-Benzoic acid, Methyl ester	248	$C_{15}H_{20}O_3$
Pentanoic acid,5-hydroxy-2,4-Di-T-Butylphenylesters	306	$C_{19}H_{30}O_3$
Pentanedioic acid(2,4-Di-T-Butylphenol) Mono-ester	320	$C_{19}H_{28}O_4$
Phenol-2-(1,1-Dimethylethyl)-4-(1,1,3,3-Tetramethylbutyl)-	262	$C_{18}H_{30}o$
2,4,6-Cycloheptatrien-1-One,3,5-Bis-Trimethylethylsilyl	250	$C_{13}H_{22}OSi_2$
1,2-Benzenediol,3,5-Bis(1,1-Dimethylethyl)	222	$C_{14}H_{22}O_2$
Silicic acid, Diehtyl-Bis-(1,1-dimethylsilyl)ester	296	$C_{10}H_{28}O_4Si_3$
Cyclotrisiloxane,Hexamethyl	222	$C_6H_{18}O_3Si_3$
Trimethyl[4-(1,1,3,3-Tetramethylbutyl)phenoxy]silane	278	$C_{17}H_{30}OSi$
Methyl-3-Bromo-1-Adamantaneacetate	286	$C_{13}H_{19}O_2Br$
1,2-Bis(Trimethylsilyl)Benzene	222	$C_{14}H_{22}O_2$

ISSN: 0975-8585

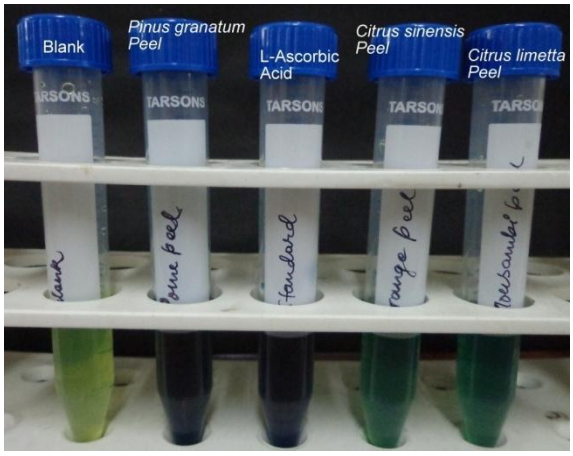

Figure 2: Reducing Power Assay for Antioxidant Activity

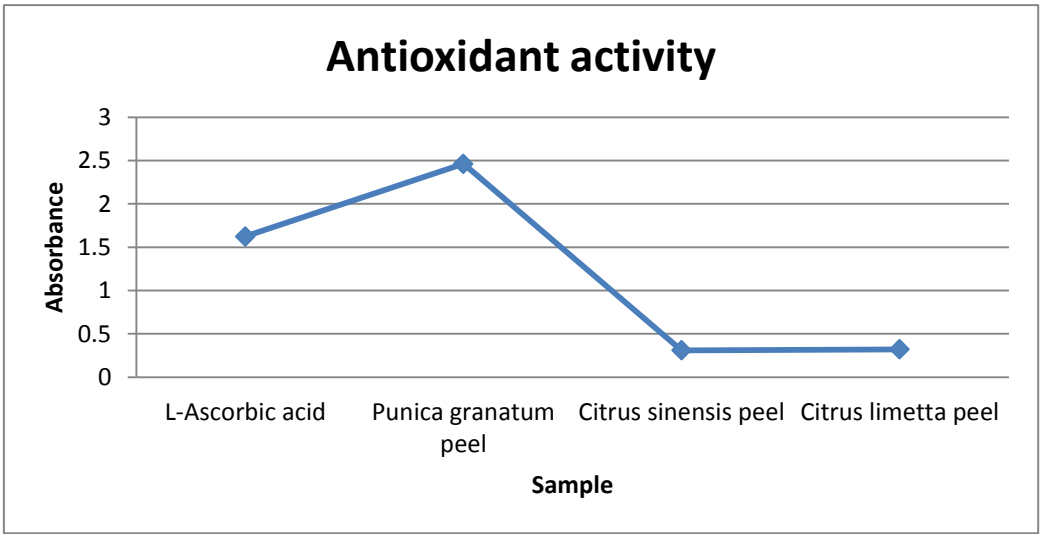

Figure 3: The Absorbance value at 700nm for the samples in study.

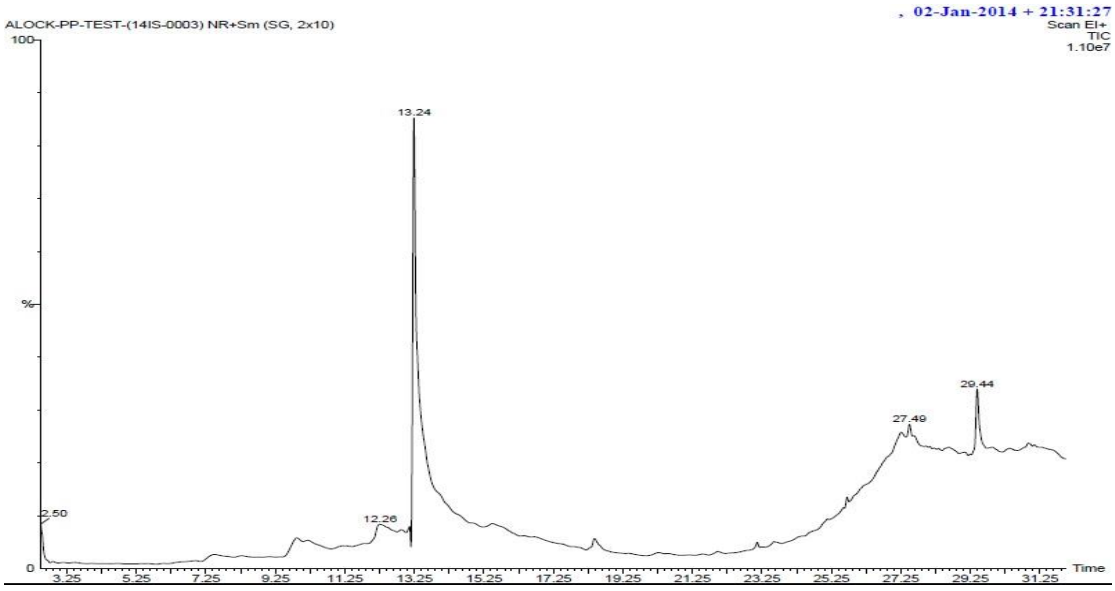

Figure 4: Gas Chromatogram of the Pinus granatum rind extract.

ISSN: 0975-8585

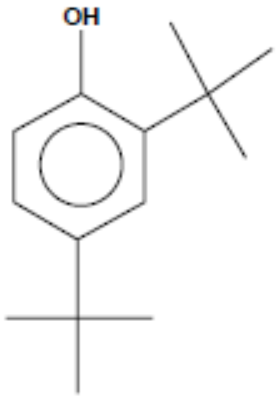

Figure 5: Phenol-2,4-Bis(1,1-Dimethylethyl)-

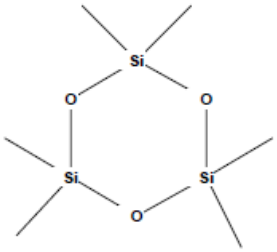

Figure 6: Cyclotrisiloxane,Hexamethyl-

Further, the Gas Chromatogram in the Figure 4 shows the presence of various phenolic and cyclic compounds in *Pinus granatum* rind extract. These compounds are mentioned in Table 1. The most abundant of the compounds detected in *Pinus granatum* extract are the Phenol-2,4-Bis(1,1-Dimethylethyl)- and Cyclotrisiloxane,Hexamethyl shown in Figure 5 and 6 respectively. These cyclic compounds are unsaturated and hence play a major role in the free radical scavenging. The degree of unsaturation in these cyclic compounds are greater than that of the enediol grouping (-COH=COH-) present in L – Ascorbic acid, making the former a better antioxidant agent than the later.

DISCUSSION

The GC-MS analysis validates the information obtained by the Reducing Power Assay for the antioxidant activity, by providing the molecular information of the sample. The phenolic compound identified in the *Pinus granatum* rind extract is supposed to be the reason behind its potent antioxidant activity. Further microbiological and analytical studies need to be done on the *Pinus granatum* rind extract for its health effects and other health related attributes.

ACKNOWLEDGEMENT

We are highly thankful to our honorable chancellor, **Dr. G Viswanathan** for the immense infrastructural support during the research works and constant support from our vice presidents and Vice chancellor without which the work would not have been successful. We also thank the VIT SIF Lab, SAS, Chemistry Division for NMR and GC-MS Analysis.

ISSN: 0975-8585

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