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NOTE

Antioxidant Activity and Brine Shrimp Lethality of *Punica granatum* Fruit Rind†

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Punica granatum fruit rind extracts were screened for antioxidant activity and brine shrimp lethality assay. All the extracts except hexane extract have shown more potent antioxidant activity compared to BHT, BHA, vitamin C and E. In addition, methanol and aqueous methanol extracts exhibited moderate brine shrimp lethality.

Key Words: *Punica granatum*, Superoxide, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), Free radical, Antioxidant activity, Brine shrimp.

Punica granatum L. (Fam: Punicaceae) is a small tree which grows up to 5-10 m height, whose leaves are oblong or obovate and fruits are globose. It is native of Iran, Afghanistan and Baluchistan, found growing wild and cultivated through out India[1]. The fruit rind is orally used for tapeworm infestations and opportunistic intestinal worms. It is also used as astringent, for treating chronic diarrhea and advanced stages of dysentery and as an abortive. Topically used as a gargle for sore throat and to treat hemorrhoids[2]. *P. granatum* fruit rind showed antioxidant[3] and antibacterial[4] activities. *P. granatum* fruit rind contains wax, resins, mannitol, non-crystallized sugars, gums, inulin, mucilage, tannin, calcium oxalate, gallic acid[1] and granatin B[5].

Free radicals and their metabolites, which are formed in the body as a consequence of normal metabolic reactions, exposure to pollutants and UV radiation, are recognized for their contribution to tissue injury and degenerative diseases, including arthritis, hemorrhagic shock, atherosclerosis, diabetes, hepatic injury, aging, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, tumor promotion and carcinogenesis[6,7]. The present study was undertaken to evaluate free radical scavenging activity of *P. granatum* fruit rind extracts through various mechanisms and to test brine shrimp lethality. The other objective of the study is to identify potent antioxidant fraction using bio-activity guided fractionation.

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Fruit rind was procured from Kondapalli forest range, Krishna District, Andhra Pradesh, in February 2003 and identified by Dr. K. Narasimha Reddy. A voucher specimen (No. 1104) is deposited in the raw drug specimen depository of the Taxonomy division at Laila Impex Research Centre, Vijayawada, India.

Extraction: Powdered material (500 g) of *P. granatum* was supplied by Laila Impex, was extracted with hexane (1.25 L), ethyl acetate (1 L), methanol (1 L) and aqueous methanol (70 %, 1 L) using a Soxhlet apparatus and the extracts were concentrated, independently, under reduced pressure to obtain 38.7, 13.1, 50 and 38.5 g crude residues, respectively.

Commercial fruit rind extract of *P. granatum* and podophyllotoxin were generously provided by Laila Impex.

Studied activities: Free radical scavenging activity was studied through various mechanisms such as superoxide and DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activities.

Superoxide radical scavenging activity was determined by nitro blue tetrazolium (NBT) riboflavin photo reduction method of McCord and Fridovich[8]. The assay mixture contained EDTA (6.6 mM) containing NaCN (3 µg), riboflavin (2 µM), NBT (50 µM), test substance and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 mL. The optical densities at 560 nm were measured before and 15 min after illumination.

DPPH radical scavenging activity was determined by the method of Lamaison *et al.*[9], which is based on the reduction in optical density of coloured methanolic solution of the DPPH free radical at 517 nm. Per cent inhibition was calculated by comparing absorbance of test substance with that of control.

Brine shrimp lethality was tested according to the method of McLaughlin *et al.*[10,11], wherein the procedure involves hatching *Artemia salina* cysts in a conical shaped vessel (1 L), filled with sterile artificial seawater (sea salt 38 g/L and adjusted to pH 8.5) under constant aeration for 48 h. After hatching, 10 nauplii were placed in each vial containing various concentrations of test drug solutions and maintained at 30 ºC for 24 h under the light of incandescent lamps and the surviving larvae were counted and the percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. LC_{50} values were obtained from the plot drawn with concentration (µg) verses percentage inhibition. Podophyllotoxin was used as a positive control (Table-1).

Conclusion

Aqueous methanol, methanol and ethyl acetate extracts of *P. granatum* fruit rind have shown potent antioxidant activity as determined by superoxide and DPPH free radical scavenging activities when compared with known antioxidants like vitamin C and E, BHT and BHA. Methanol extract and aqueous methanol extracts of *P. granatum* has also exhibited moderate and dose dependent brine shrimp lethality compared with podophyllotoxin.

TABLE-1
ANTIOXIDANT ACTIVITY AND BRINE SHRIMP
LETHALITY OF *P. granatum* FRUIT RIND EXTRACTS*

Test substance	Antioxidant activity (IC_{50} values in µg/mL)		Brine shrimp lethality (LC_{50} values in µg/mL)
	Superoxide	DPPH	
PG AqMeExt	4.80	3.90	75.0
PG MeExt	6.70	6.00	72.0
PG EtOAcExt	12.50	9.50	> 200
PG HexExt	> 100	> 100	> 200
PG Commercial extract	8.50	10.00	157
Vitamin-C	145	3.75	–
Vitamin-E	308	> 1000	–
BHT	105	4.60	–
BHA	169	3.95	–
Podophyllotoxin	–	–	4.2

*Values are mean of three determinations.

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