

Standardization of Unani Drug Post-e-Anar (Fruit rind of *Punica granatum* L.)

*Nazish Siddiqui,
M. Masihuzzaman Ansari,
Alvia Khan,
Mohd. Bilal Tafseer,
Saba Viqar
and
Abdul Haleem

Department of Ilmu Advia,
A.K. Tibbiya College,
Aligarh Muslim University,
Aligarh 202002

Abstract

The peel of fruit or fruit rind of *Punica granatum* L. is used as herbal drug. In Unani medicine it has been used to cure many ailments and known as Post-e-Anar. It is anti-oxidant, anti-cancerous, anti-inflammatory and anti-allergic. But its standardization has not been done so far, so keeping in mind the medicinal properties of Post-e-Anar, its diagnostic characters has been identified by following the modern scientific quality control procedures. The study provides organoleptic, physico-chemical and thin layer chromatographic profile which will be helpful in laying down the standards and pharmacopoeial parameters. The qualitative screening for phytochemicals was carried out to facilitate desirable therapeutic efficacy. Besides this, novel Infra-red (IR) spectral study of rind extract has also been conducted by recording the IR spectrum of the extract, which can be used as fingerprints for identity of drug Post-e-Anar.

Keywords: Standardization , Post-e-Anar, Physico-chemical, IR Spectrum, Fruit rind, Phytochemicals.

Introduction

The plant *Punica granatum* L. is a large deciduous shrub or a small tree (Anonymous, 2009). It belongs to the family Punicaceae. It is commonly known as Anar and considered to be native of Iran, Afghanistan and Baluchistan. It is also found growing wild in the Himalayas between 900 to 1800 m and also cultivated throughout India. Flowers occur during April to July, while fruiting takes place during July to September (Anonymous, 2007). Various parts of plants are used as drug. Fruit juice is excellent for curing diarrhoea, dysentery and other ailments such as colitis, anaemia, jaundice, high blood pressure, piles and arthritis (Anonymous, 2009; Dipak *et al.*, 2012), decreases cholesterol absorption (Julie Jurenka, 2004). The leaf, fresh seeds and root bark of *Punica granatum* have been studied and standardized. (Anonymous, 2009; Anonymous, 2007; Anonymous, 1997). The external leathery cover of fruit of *Punica granatum* L. is known as rind and in Unani medicine it is known as Post-e-Anar. The rind or peel of fruit contains allergic acid as one of the main constituents which interrupts free radicals induced damage and acts as anti-oxidant (Yunfeng *et al.*, 2006; Amir *et al.*, 2011). It also acts as anti-cancerous inhibiting the growth of tumours and is useful in cancer of colon, breast, prostate, skin and pancreas (Julie Jurenka, 2004; Amir *et al.*, 2011; Negi *et al.*, 2011). Besides this rind extract has been found to possess anti-allergic and anti-inflammatory activities also (Panichayupakaranant *et al.*, 2010). The world is heavily polluted with bacteria,

*Author for correspondence

viruses and fungi infections and these are the major cause of diseases in human beings. Further, due to indiscriminate use of synthetic anti-microbial drugs, most infective microorganisms have developed resistance to many antibiotics. Thus, there is an urgent need to discover novel herbal anti-microbials, as they have less or no side effects. The fruit rind extract of pomegranate also shows significant antibacterial and antifungal activities (Panichayupakaranant *et al.*, 2010; Negi *et al.*, 2003; Adollahzaden *et al.*, 2011; Dahham *et al.*, 2010), Apart from this, Post-e-Anar extract was found to exhibit antimalarial activity (Aqli *et al.*, 2010), It is also useful for prevention of cardio-vascular diseases, diabetes, alzheimer's disease, arthritis, obesity, dental problem (Mohammad & Kashani, 2012; Negi *et al.*, 2003) and possesses antidiarrhoeal activity (Akter *et al.*, 2013) . Thus, keeping in mind the medicinal importance of drug, various physico-chemical and phytochemical studies on the fruit rind of *Punica granatum* (Post-e-Anar) were carried out with a view to standardize it and lay down standards for its purity, quality control & quality assurance, which has not been taken earlier.

Methodology

Collection of Plant Material

Red pomegranate fruits were collected . Longitudinal cuts were given to the peel of the fruit and peels were gently removed with the help of hand and knife. The seeds were removed and peel was cut into pieces of 1 inch size. It was then placed in a sunny spot during day light hours every day till the peel gets hard and loses all the moisture. Dried peels were powdered to get 60-mesh size using mixer grinder and placed in air tight container as 'Post-e-Anar' for study.

- (i) Organoleptic Parameters: The colour, taste, odour, solubility were noted which provide first hand information.
- (ii) Physico-chemical Parameters: Various physico-chemical studies like total ash, acid insoluble ash, water soluble ash, successive extractive values using soxhlet extraction method, water and alcohol soluble matter, pH, bulk density, moisture content were carried out as per guidelines of WHO and others (Anonymous, 1998; Anonymous, 1963),
- (iii) Qualitative Analysis of Post-e-Anar: was conducted to identify the organic chemical constituents present in the drug (Overtone, 1963).
- (iv) IR Spectroscopic Study: For this alcoholic extract of the drug was obtained by refluxing powdered drug (5.0 g) with absolute alcohol (50 ml) for 5 hrs and removing the solvent under reduced pressure. The IR spectrum of alcoholic extract was determined in KBr pellets with Perkin Elmer 1600 FTIR spectrometer.

- (v) Thin layer chromatographic analysis: For this ethyl acetate and ethanolic extract was conducted^{20,21} in percolated silica gel 60F₂₅₄ TLC plates. The plates were visualized in day light and UV short wavelength respectively (Harborne, 1973; Stahl, 1969).

Observations and Results

Organoleptic characters: The powder of the fruit rind of pomegranate was brownish yellow with characteristic odour and taste, showed good solubility in water (Table 1).

Physico-chemical constants: Different physico-chemical constants were determined three times and their average values are depicted (Table 2).

Table 1 : Organoleptic characters of fruit rind of *Punica granatum* L.

S.No.	Parameters	Observations
1	Colour	Brownish yellow
2	Odour	Characteristic
3	Taste	Characteristic
4	Solubility	Good in water

Table 2 : Physico-chemical Constants of fruit rind of *Punica granatum* L.

S.No.	Parameters	Analytical values (%)*
1	Ash Value	
	Total Ash (w/w)	4.21
	Acid Insoluble Ash (w/w)	2.21
	Water Soluble Ash (w/w)	2.9
2	Solubility	
	Alcoholic Soluble Matter (w/w)	7.0
	Water Soluble Matter (w/w)	8.8
3	Successive Extractives	
	Petroleum ether	0.53
	Diethyl ether	5.0
	Chloroform	0.49
	Ethyl acetate	1.73
	Absolute alcohol	32.62
	Distilled water	25.88
4	pH Values	
	1% Aqueous solution	3.73
	10% Aqueous solution	3.40
5	Moisture content (v/w)	12
6	Bulk density	0.49

*Values are average of three experiments

Qualitative analysis of Post-e-Anar: The phytochemicals present in the drug were qualitatively analysed by different chemical tests and results are given (Table 3).

IR spectral study of the drug: Novel IR spectral study of the alcoholic extract of the drug was done by running the alcoholic extract in the IR range (4000-667cm⁻¹) of the electro-magnetic spectra and major characteristic peaks were noted (Fig. 1, Table 4).

Thin layer chromatographic profile: Thin layer chromatographic analysis of the ethanolic and ethyl acetate extract of Post-e-Anar was carried out using Benzene : methanol : acetic acid (45 : 8 : 4) and ethyl acetate : acetic acid (20:4) respectively as solvent system. Rf values were calculated after the development of chromatogram. The Rf values in the given solvent are used to characterize the drug's identity and purity. The results obtained are given in table-5.

Table 3: Qualitative Analysis of Phytochemicals of fruit rind of *Punica granatum* L.

S.No.	Chemical Constituents	Test / Reagents	Inference
1.	Alkaloids	Dragendorff,s reagent Wagner,s reagent Mayer,s reagent	+ve +ve +ve
2.	Carbohydrates	Molish Test Fehling Test Benedicts Test	+ve +ve +ve
3.	Flavonoids	Mg ribbon and dil Hcl	+ve
4.	Glycosides	NaOH Test	+ve
5.	Tannins / Phenols	Ferric chloride Test	+ve
6.	Protein	Xanthoproteic Test Biuret Test	+ve +ve
7.	Steroids / Terpenes	Salkowski Reaction	+ve
8.	Amino acids	Ninhydrin Solution	-ve

Table 4: IR Spectral Details of alcoholic extract of fruit rind of *Punica granatum* L.

Region of electromagnetic spectra	Characteristic peaks
IR, γ (cm ⁻¹)	3412, 3329, 1706, 1615, 1512, 1448, 1345, 1222, 1190, 1048

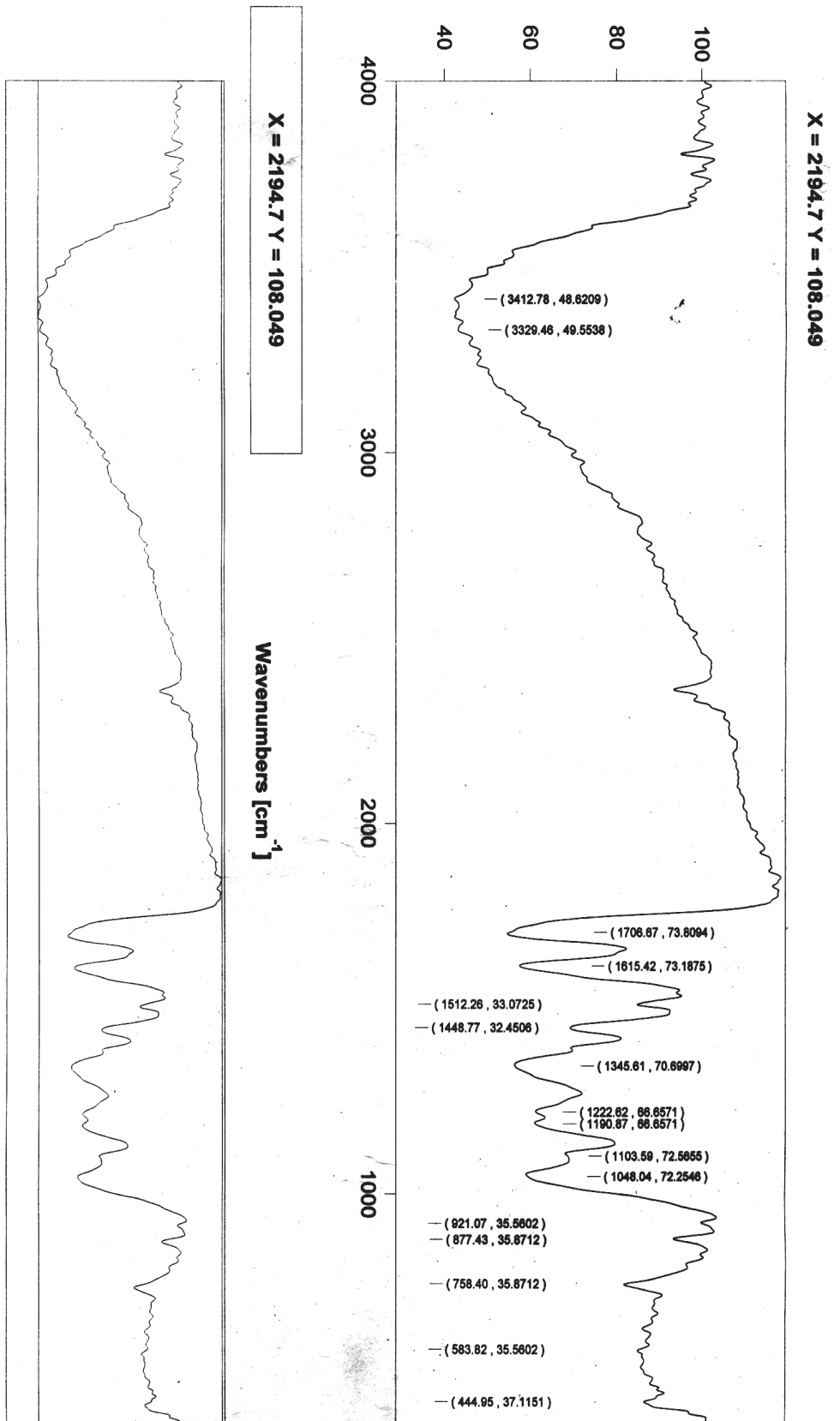


Figure 1

Table 5 : TLC Profile of alcoholic extract of fruit rind of *Punica granatum* L.

Extract	Solvent System	Visualizing Agent	No. of Spots	Rf Value
Ethyl Acetate	Ethyl Acetate : Acetic Acid (20:4)	Day Light	3	0.41, 0.65, 0.76
Ethanol	Benzene : Methanol : Acetic Acid (45:8:4)	UV Short wave	3	0.47, 0.59, 0.88

Discussion

The interest in evaluating therapeutic effects of plants have increased as 80% of world's population depends on complementary and alternative medicine for their health care needs (Magee, 2005; Duraipandiyar, 2006). The quality of herbal drugs is sum of all the factors which contribute directly or indirectly to its safety, effectiveness and acceptability of the product. Standardization of drugs means confirmation of its identity, determination of its quality and purity (Ekka, 2008). Initially organoleptic studies were used to authenticate the drugs but some drugs require other physical and chemical studies too. So standardization of Post-e-Anar was carried out as it has an important role in folk medicine. The present standardization study has brought out many diagnostic characters of herbal drug Post-e-Anar (fruit rind of *Punica granatum*). Organoleptic characters provide preliminary information regarding colour, taste, odour and solubility which is necessary for authentication. Physico-chemical parameters were determined to ascertain the identity and purity. Ash values were found as total ash 4.21%, acid insoluble ash 2.21% and water soluble ash 2.9%, if the drug is adulterated with siliceous or earthy matter ash values may change. Moisture content has been determined by toluene distillation method as 12%. If its value increases that may provide an evidence that the drug is not dried and stored properly. The pH value of 1% and 10% aq. solution was 3.73 and 3.40 respectively, which helps in knowing the drug receptor site interactions and also stability and therapeutic activity of the drug. The amount of extract that a drug yields to a particular solvent is an approximate measure of the amount of certain constituents or group of related constituents that the drug contains (Jenkins *et al.*, 1967). Thus extractive values of Post-e-Anar in six organic solvents were calculated as an index of its purity, and these are shown in Table 2. Phytochemical screening of drug was done to know the chemical constituents present in Post-e-Anar (Table 3).

Another important technique taken here to confirm the identity was Infra red (IR) spectroscopy. The IR spectrum of alcoholic extract of fruit rind of *Punica granatum* (as Post-e-Anar) was recorded in fig-1, table-4. Initially the IR spectroscopic method was restricted only for structural elucidation of isolated compounds from the herbal matrix but now it is also useful in phytochemical studies as a “fingerprinting” device for comparing a natural sample with synthetic sample. In IR spectrum, region 1430-910 cm^{-1} is known as finger print region, which can be compared with the finger prints of human beings, which differs from person to person. In the same manner this region would differ from drug to drug. In addition to this IR spectrum also provides information about functional groups present in the chemical constituents of the drug. To check the purity of drug IR spectrum may be compared with the authentic sample. If the characteristic bands are similar and identical, the test drug would be genuine. Apart from this thin layer chromatographic (TLC) study was also carried out. Now a days other instrumental chromatographic methods are also used like GC & HPLC but TLC provide first characteristic fingerprints of herbs and various pharmacopoeias still use TLC (Jain and Sharma, 2005). TLC was made here to construct the fingerprint of Post-e-Anar, as it is simple, versatile, sensitive and economic. Results are summarized in table-5. This study assumes great significance as it will provide a key of diagnostic characters which will serve as an important tool in laying down the standards for quality assurance.

Acknowledgement

The authors are grateful to the Chairman, Department of Ilmul Advia, AMU, Aligarh, for providing necessary research facilities. Facilities provided by SAP (DRS-1) by way of generous research support are duly acknowledged.

References

- Adollahzaden, S., Mashouf, R.Y., Mortazavi, H., Moghaddam, M.H., Roozbahani, N. and Vahedi, M. , 2011. Antibacterial and antifungal activity of *Punica granatum* peel extract against oral pathogens, *J Dent* (Tehran), 8(1): 1-6.
- Agli, M.D., Galli, G.V., Bulgari, M., Basilico, N., Romeo, S., Bhattacharya, D., Taramelli, D. and Bosisio, E., 2010. Ellagitannins of fruit rind of Pomegranate (*Punica granatum* L) antagonize in vitro the host inflammatory response mechanisms involved in the onset of malaria, *Malaria Journal*, 9: 208.
- Akter, S., Sarker, A. and Hossain, M.S., 2013. Antidiarrhoeal activity of rind of *Punica granatum*, *International Current Pharmaceutical J*, 2(5): 101-104.
- Amir, A., Motaal, Shaker, S., 2011. Anticancer and antioxidant activities of standardization of whole fruit, pulp and peel extract of Egyption pomegrate, *The Open Conference Proceedings Journal* 211(41): 41-45.

- Anonymous , 2009. Unani Pharmacopoeia of India, Part I, Vol. VI. Department of Ayush, Govt. of India, New Delhi, pp. 9-10.
- Anonymous, 2007. Unani pharmacopoeia of India, Part I Vol II D/O Ayush govt of India ministry of health and family welfare New Delhi, pp. 5-6.
- Anonymous, 2008. Quality control manual for Ayurveda, Siddha and Unani medicine, Govt of India, Dept of AYUSH, New Delhi, pp. 21-29.
- Anonymous, 1997. Standardization of single drugs of Unani Medicine, Part III. CCRUM, New Delhi, pp. 250-254.
- Anonymous, 1998. Quality control methods for medicinal plant materials. World Health Organization, Geneva, pp. 25-28.
- Dahham, S.S., Ali, M.N., Tabassum, H. and Khan, M., 2010. Studies on antibacterial and antifungal activity of pomegranate (*Punica granatum* L), *American-Eurasian J. Agric & Environ. Sci.* 9(3): 273-281.
- Dipak, G., Patel, A., Chakraborty, M. and Kanath, J.V., 2012. Physiochemical & pharmacological profile of *Punica granatum* L.: An overview, *Int. Res. J. Pharmacy* 3(2): 65-68.
- Duraipandiyan, V., Ayyanar, M. and Ignacimuthu, S., 2006. Antimicrobial activity of some ethanomedicinal plants used by paliyar tribe from Tamilnadu India, *BMC complement Altern Med* 6: 35.
- Ekka, N.R., Namdeo, K.P. & Samal, P.K., 2008. Standardization strategies for Herbal drugs- An overview, *Research J. Pharm. and Tech* (4): 310-312.
- Growther, L., Sukirtha, K., Savitha, N. and Niren, Andrew, S., 2012. Antibacterial activity of *Punica granatum* peel extracts against shiga toxin proceeding *E.coli*, *Int Journal of Life Science, Biotechnology and Pharma Research* 1(4): 164-172.
- Jain, M.K. and Sharma, S.C., 2005. Modern Organic Chemistry. Vishal Publishing Co, India, 1038.
- Jenkins, G.L., Knevel, A.M. and Digangi, F.E., Quantitative pharmaceutical chemistry. Mcgraw Hill Book Company, 6th Ed, London, pp. 239-280.
- Julie Jurenka, M.T., 2004. Therapeutic application of pomegrate (*Punica granatum* L): A review, *Alternative Medicine Review* 13(2).
- Magee, K.A., 2005. Herbal Therapy: A review of potential health risks and medicinal interactions, *Orthod Craniofac Res.* 8(2): 60-74.
- Mohammad, S.M. and Kashani, H.H., 2012. Review: Chemical composition of the plant *Punica granatum* L and its effect on heart and cancer, *Journal of Med Plant Res*, 6(40): 5306-5310.

- Negi, P.S., Jayaprakash, G.K. & Jena, B.S., 2003. Antioxidant and antimutagenic activity of pomegrate peel extracts. *Food Chemistry* 80 (3): 393-397.
- Negi, P.S., Jayaprakash, G.K. & Jena, B.S., 2003. Antioxidant and antibacterial activity of pomegrate peel extracts. *J of Food Sci.* 68(4): 1473-1477.
- Overtone, K.H., 1963. Isolation, purification & preliminary observation in elucidation of structures by physical & chemical methods. Bentley Interscience Pub, New York, pp. 34.
- Panichayupakaranant, P., Tewtrakul, S. and Yuenyongsawad, 2010. Antibacterial, anti-inflammatory and antiallergic activity of standardized pomegranate rind extract. *Food Chemistry*, 123(2): 400-403.
- Stahl, 1969. Thin layer chromatography: A Laboratory Handbook. Springer Verlag Student edition, Springer Verlag, Berlin, pp. 52-86,127-128,900.
- Wagner, A., Blatt, S., Rickl, V., 1996. Plant Drug analysis, A thin layer chromatography Atlas, Springer Berlin, p. 2.
- Yunfeng, Li., Changiang, G., Yang, J., Wei, J. and Xu, J., 2006. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegrate pulp extract. *Food Chemistry* 36(2): 254-260.

