ejpmr, 2018,5(7), 271-275

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ISOLATION, PHYTOCHEMICAL SCREENING AND BIOLOGICAL EVALUATION OF PUNICA GRANATUM LEAVES EXTRACT

P. J. Shirote Deepali S. Sul and *Sheela S. Thorat

India.

***Corresponding Author: Sheela S. Thorat**

India.

Article Received on 25/04/2018 **Article Revised on 15/05/2018** **Article Accepted on 05/06/2018**

ABSTRACT

The Punica granatum family Punicaceae Synonym- Punica florida salib, Punica nana L. The tree is native from Eran to the Himalayas in northen India. the chemical constituent consist of flavanoids, tannin, alkaloid, phenolic compounds, ellagic acid. These are used as anti-inflammatory, antiulcer, nephroprotective, antidiabetic. The chemical constituent was identified by phytochemical investigation and isolated by thin layer chromatography and column chromatography. The constituent was analysed by spectral analysis and structure was identified. the ethanolic extract of leaves of Punica granatum showed significant diuretic activity.

KEYWORDS: Punica granatum, tannin, diuretic.

INTRODUCTION

The Punica granatum Family Punicaceae Synonym- Punica florida salib, Punica nana L. The punica granatum tree is native from Eran to the Himalayas in northen India and it has been cultivated in region of Asia, Africa and Europe. The transverse section of leaf of Punica granatum L. showed presence of upper and lower epidermis. The anomocytic and anisocytic stomata were present in epidermis.

Chemical constituent and Uses

Table no. 5.3- Chemical constituents and uses.

Plant part	Chemical constituent	Uses
Whole plant	Flavonoids, antocyanin, β-carotene, phenolic compound, ellagic acid.	Antiinflammatory, antiulcer, Antidiabetic, antioxidant, anticonvulsant, antidepressant.
Seed	Punicic acid, ellagic acid, sterols.	Nephroprotective, antioxidant, Antidiabetic.
Root and bark	Ellagitannin (punicalin and punicalagin), piperidine alkaloid.	Molluscicidal, antihelmintic.
Leaves	Tannins(punicalin and punicafolin), flavones glycosides(luteolin, apigenin)	Antioxidant, antiobesity.
Pericarp (peel, rind)	Phenolic compound, punicalagin, gallic acid, ellagic acid, quercetin, flavonols and flavones, anthocyanidins.	Antifungal, antimutagenic, antiinflammatory, antidiarrheal.
Flower	Gallic acid, ursolic acid, triterpenoids (maslimic and Asiatic acid)	Antioxidant, hepatoprotective.

MATERIAL AND METHODS

Extract used

Ethanolic exract of leaves of Punica granatum L.

Experimental

Collection of plant -The fresh leaves of Punica granatum L. were collected from local region Authentication of plant -Authentication of leaves of Punica granatum were done at willingdon college. Distillation of solvent- Distillation of solvent done before extraction of plant material.

Method of extraction

Soxhlet extraction

Preparation of Extract -The collected leaves of plant were washed with water and dried under shade, after drying of plant material it was powdered by mixer grinder. the 100 gm of powder extracted with Ethanol in

soxhlet extractor at temperature of 40 degree C. The extraction was continued until solvent in the thimble become clear. these extract was stored in dessicator and used for further phytochemical study. the preliminary phytochemical study was performed.

Preliminary phytochemical investigation
Chemical Test Result
1. Test for glycosides +
2. Test for Carbohydrate's +
3. Test for Flavonoid's +
4. Test for Alkaloid's +
5. Test for tannin's +

Physiochemical evaluation of extract was performed by different method such as the total ash value and acid insoluble ash value, water soluble ash value and total moisture content. this method was used to remove the adulteration from the extract.

Isolation of ethanolic extract of *Punica granatum.*
Isolation of punica granatum leaves extract carried out by using column chromatography. Mobile phase used as Petroleum ether: Ethyl acetate: Isopropyl alcohol: Methanol (8:1:3 drops:2). The isolated fraction were collected, dried and used for further study. Isolated fraction were identified by phytochemical test, TLC and melting point.

- **Physical and chemical test for isolated fraction**

Table no. 7.3 physical and chemical test of isolated fraction.

Physical evaluation		
Test	**Observation**	**Inference**
Melting point	194^0C	Tannin (punicalin) might be present
Chemical evaluation		
Isolated fraction + 5% ferric chloride solution	Deep blue-black colour	Tannin present
Isolated fraction + lead acetate.	White precipitate	Tannin present

Experimental
Invivo Diuretic activity: Evaluation of diuretic activity of plant extract on rat by using, **LIPSCHITZ test model.**

A method used for testing diuretic activity in rats has been described by LIPSCHTZ etal, (1943), the test is based on water and sodium excretion in test animals and compared to rats treated with a high dose of urea. The lipschtz- value is the quotient between excretion by test animal and excretion by urea control.

MATERIAL AND METHODS
Drug – frusemide, Chemicals – sodium, potassium, Extract used – ethanolic extract of punica granatum leaves, Equipment – flame photometer, pH meter.

Test animals: Wistar albino rat of both sex and weighing 100-200gm were used for diuretic activity. The animals were kept under controlled conditions (temperature 25 ± 0.5^0c. light/dark cycle, 12/12h).

Preparation of extract for animal study: The crude ethanolic extract at dose 250 and 500 mg/kg body weight were selected and Rats were divided into four groups of six animals in each group. 15 hr prior to the experiment food are withdrawn.

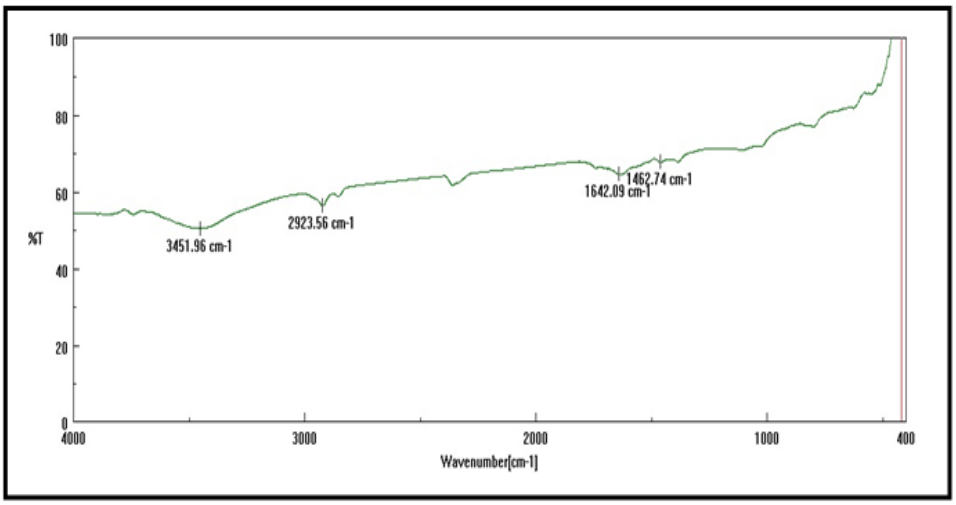
Group I – group I served as control group received water orally.
Group II – group II served as standard group received frusemide orally at dose 10mg/kg body weight.
Group III – group III served as test group received orally EEPG at dose 250mg/kg body weight.
Group IV – group IV served as test group received orally EEPG at dose 500mg/kg body weight.

After administration of dose to the animal were placed in metabolic cage provided with wire mesh bottom and funnel to collect urine. Stainless steel sieves are placed in funnel to retain faecal matter and avoid cross contamination of urine with faecal matter. urine was collected after drug administration at 5hr and 24 hr.

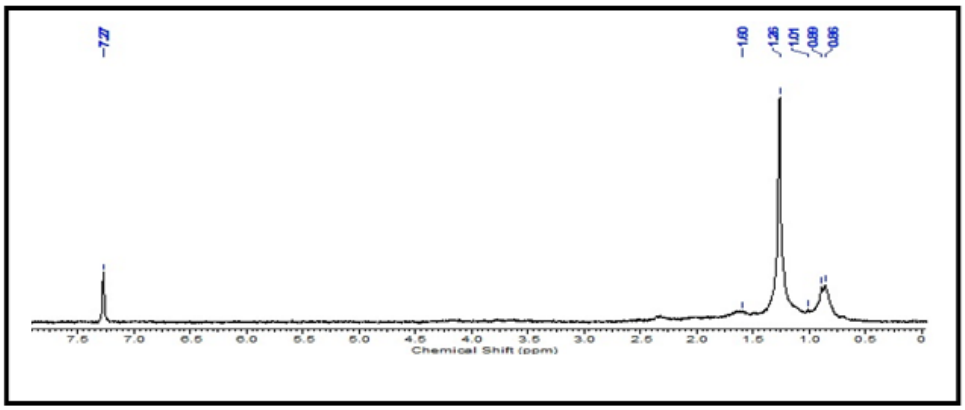
Biochemical Estimation
1) Total urine volume 2) Concentration of sodium and potassium 3) pH of urine.

RESULT AND DISCUSSION

The IR spectra obtained from A.B.C.P.Sangli.



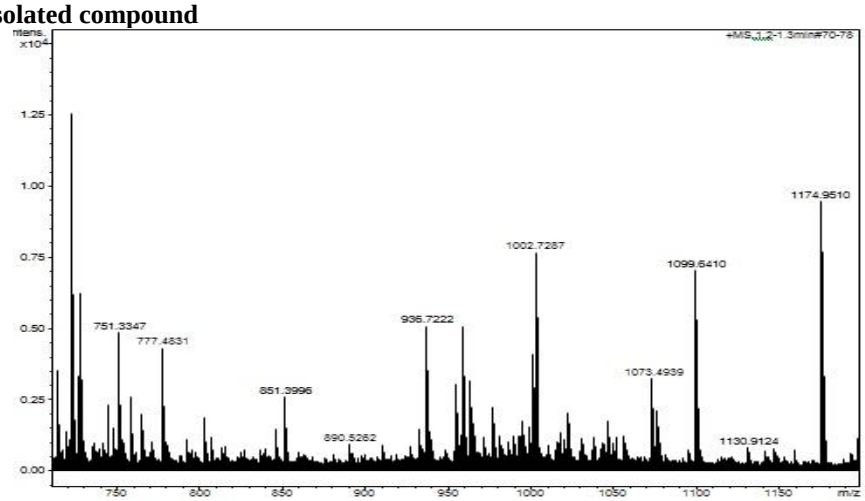
IR spectra of isolated tannin compound



NMR of isolated compound

Sr no.	Value (ppm).	Indication.
1	7.27	Aromatic proton
2	1.26	Alkyl proton
3	0.89,1.0	Alkyl proton

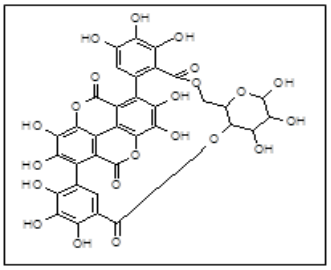
Mass spectra of isolated compound



Mass spectra obtained from Pune University
The IR spectra of compound shows absorption band of O-H str.at 3451.96, the C-H str.at 2923.56, C=O str.at 1642.09 and O-H bending at 1462.74. All these absorption band shows presence of tannin.
The NMR spectra shows chemical shift at 7.27 shows presence of aromatic ring, also chemical shift at 1.26 shows presence of alkyl proton.

The Mass spectra of obtained compound showed the molecular weight 782.52 and molecular ion peak 777.48.

From these spectral analysis we were concluded that the resulting compound was Punicalin which was tannin in nature.



Structure of Punicaline

Pharmacological activity

Table no. 7.19 Diuretic effect of ethanolic extract of *punica granatum*.

Groups	Dose (mg/kg)	Volume of urine (ml/5hr)	Volume of urine (ml/24hr)
Control	-	1	1.2
Frusemide(standard)	10mg/kg	5	6.6
PG1(test extract1)	250mg/kg	1.8	2.8
PG2(test extract2)	500mg/kg	5.4	6.4

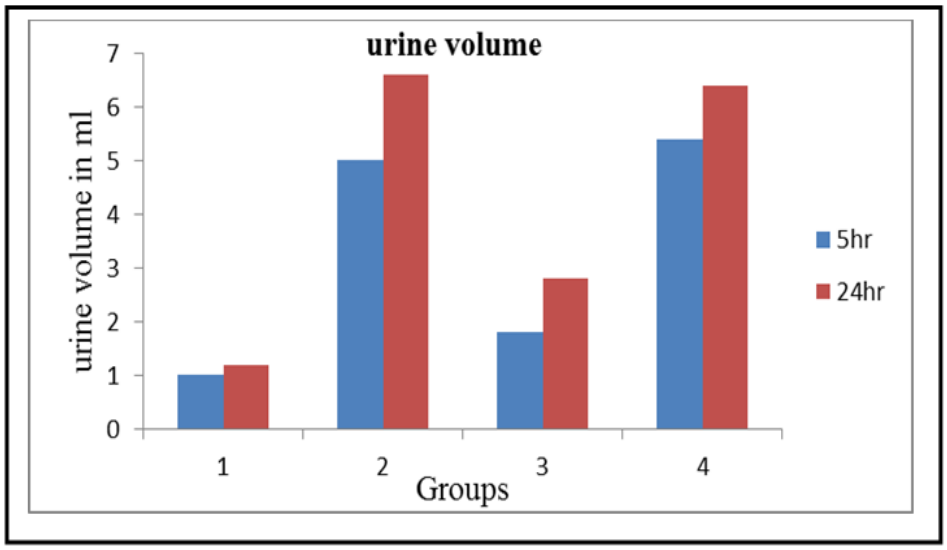


Fig. 7.22: Effect of ethanolic extract of *punica granatum* on urine output.

Groups	Urine pH	Urine sodium (ppm)	Urine potassium (ppm)
Control	7	448.2 ± 0.9	24.2 ± 0.1
Frusemide(standard)	7.7	568.1**** ± 1.07	47.0**** ± 0.035
PG1(test extract1)	6.8	516.0**** ± 0.55	32.1*** ± 0.6
PG2(test extract2)	7.2	539.4**** ± 0.3	41.7**** ± 0.7

The result are mean ± SEM, n=6, p<0.0001compared to control.

CONCLUSION

From phytochemical evaluation it was found that presence of chemical constituent and by thin layer chomatograpgy and column chromatography these chemical constituent was separated and identified by spectral analysis. From that it was concluded that the presence of punicaline which was tannin in nature.

ACKNOWLEDGEMENT

The authors thankful to the Department of Pharmaceutical Chemistry and Appasaheb Birnale College of Pharmacy, Sangli to providing necessary facilities to complete these research work.

REFERENCES

1. Ghani A, (2013), Herbal Medicines, Present status, future prospects, News Pharmabiz.com.
2. Mukharji P, (2005), 'Quality control of herbal drug', 5th edi. an approaches to evaluation of botanicals, Business Horizon Publication, 1-10.
3. Chopra R, Nagar S, Chopra I, et. al., (2006), 'Glossary of Indian medicinal plants', National Instituent of Science Communication and Information Resources, New Delhi, Reprinted, 4.
4. Rashid M, Malik I, Nizamul H, et. al., (2015), 'A comprehensive review of phytochemical and pharmacological profile of anar(punica granatum linn): a heaven fruit, Journal of Ayurvedic and Herbal Medicine, 1(1): 22-26.
5. Tripathi K.D., essential of Pharmacology, 5th edi., 2005; 525-528.
6. Jackson E, John S, Bruton L, et. al., (2006), 11th edi. Diuretics, In: Goodman and Gilman's, The pharmacological Basis of Therapeutics, 737-769.
7. Sayana S, Patil P, (2014), 'study of diuretic activity of ethanolic extract of leaves of cissampelos pareira in rats', Asian Journal of Pharmaceutical and Clinical Research, 7(4): 157-159.