Formulation and evaluation of novel herbal combination for Anti-urolithiasis activity

Pratiksha Nikam[1], Madan Pomaje[2]
[1,2]*Department of Pharmaceutics, Govindrao Nikam College of Pharmacy Sawarde -415606, Chiplun, India*

Abstract **- The presented study was being carried out to evaluate the invitro anti-urolithiasis activity of a novel combination including roots and seeds of Punarnava and Cotton seed.**

The in-vitro inhibition of commonly observed calcium-oxalate crystal in most of the urinary caliculi has been carried out by using a combination of extract by different assay procedures. Artificial urine was prepared by supersaturating with calcium oxide and urolithiasis was studied by Nucleation assay and Aggregation assay. Crystal inhibition in artificial urine was studied at different time intervals using root and seed extract at different concentrations 50, 100, 150, 200,250 mg/µl each respectively. The comparison study was carried out between the selected combination and marketed formulation Cystone (Control).

Index Terms **- Urinary calculi, Calcium oxalate, Punarnava, Artificial urine, invitro assay.**

I.INTRODUCTION

Urolithiasis (Nephrolithiasis) or kidney stone is formation of urinary calculi at any level of urinary tract. Urolithiasis is a multifactorial disease where stones are formed at the location where the urinary tract with its cause lying in series of events that lead to faction of equilibrium between promoters and inhibitors of crystallization in the urinary system. Less volume of urine, urinary pH, presence of sodium, calcium, oxalate etc. known to cause crystallization. There are many perspectives for the treatment of urolithiasis that include the use of various synthetic and natural drugs. Renal calculi can be broadly classified in two large groups: tissue attached and unattached. Calculi are mainly united by calcium oxalate monohydrate renal calculi, with a analyzed attachment site to the renal papilla and basically consisting of a core located near to the attachment site (concave zone) and radially striated concentrically laminated peripheral layers. Free calculi, with no detectable site of attachment to papilla, are developed in renal cavities of low or reduced urodynamic efficacy and can exhibit diverse composition and structures, according to reports published Randall's first description of papillary calcifications and their possible active role in the genesis of COM papillary calculi.

Stone promoters and inhibitors: -[3]

Promoters	Inhibitors
Calcium	Citrate
Oxalate	Pyrophosphates
Urate	Magnesium
Sodium	Osteopontin
Cysteine	Glycosaminoglycan's
Low urine pH	Protease inhibitor's
Tam Horsfall protein	Urine, prothrombin fragment

II. MATERIALS AND METHODS

A. Selection of plant : *Boerrhavia diffusa* (Punarnava) was selected from local areas of konkan region whereas the seeds collected from local market. Identification was done at department of Agricultural Science, Horticulture and technology(Sahyadri Shikshan Sanstha, Sawarde.

B. Extraction of plant : Roots of punarnava were sun dried , grinded to form fine powder . A sample of 50 g of dried powder was used for each extraction. The dried samples were separately Soxhlet Extracted in ethanol (100 ml/ gm dried weight) on a heating mantle for 15 to 20 cycles at the temperature of 50 ^{0}C.Each of the extracts was concentrated and re-concentrated in petroleum ether to remove fatty substances. The punarnava extract solution was cooled and filtered using Whatmann filter paper and condensed to

decreased quantity of solvent using soxhlet extractor. The extract was then dried in hot air oven at NMT 100ºC.

C. **Phytochemical analysis :** The Phytochemical analysis was carried out for presence of – Carbohydrates, Proteins, Steroids, Alkaloids, Glycosides, Flavonoids, Phenols .

CHARACTERIZATION OF EXTRACT

A Punarnava :

Appearance : Amorphous in nature

Colour : Beigh

Odour : Characteristic

Melting Point : 125.15º c

B Cottonseed oil :

Appearance : Clear oil

Colour : Light golden colour

Odour : No characteristic odour

Melting Point : 343.55º c

III.:PHARMACOLOGICAL INVESTIGATION/ IN VITRO STUDY OF ANTIUROLITHIASIS ACTIVITY : NUCLEATION ASSAY:[41]

Ingredients	Qty given for a tablet	Role
Punarnava	250 mg	Anti urolithiatic
Cottonseed oil	250 µl	Anti urolithiatic
Ginger powder	50mg	Anti emetic
Starch	10 %	Disintegrant
Lactose	110 mg	Diluent
PVP in alcohol	5%	Binder
Talc	2%	Glidant
Mg. Stearate	1%	Lubricant

In vitro antiurolithiatic assay was conducted by the procedure as mentioned in literature . The assay was carried out in the absence (control) and presence of inhibitor (standard/extract/fraction). About 5 mM and 7.5 mM calcium chloride and sodium oxalate solutions were made in a buffer composed of 50 mM Tris and 150 mM sodium chloride at pH 6.5. Stock solutions of standard (cystone) and samples (extracts of roots) were prepared at a concentration of 10 mg/ml. About 1 ml of calcium chloride was added in both the control and sample sets. Additionally, 1 ml of distilled water was added in the control set. On the contrary, 1 ml of various dilutions of sample (200, 400, 600, 800, 100 µg/ml) were added in the sample set instead of distilled water. The onset of crystallization was achieved by the addition of 1 ml of sodium oxalate solution to all the sets. The tubes were incubated at 37° for 30 min after which the absorbance was read at 620 nm using SHIMADZU UV/Vis spectrophotometer. The percentage inhibition of nucleation was calculated as follows: percent inhibition=[(C-S)/C]×100, where, C=turbidity of control set, S=turbidity of sample set.

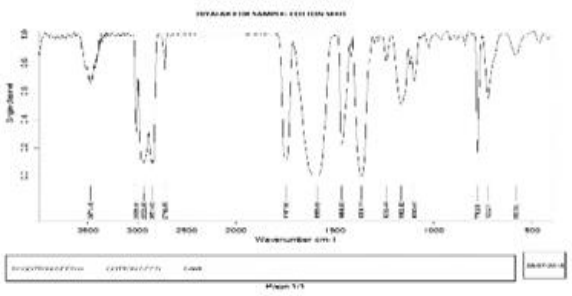
B AGGREGATION ASSAY: [42]

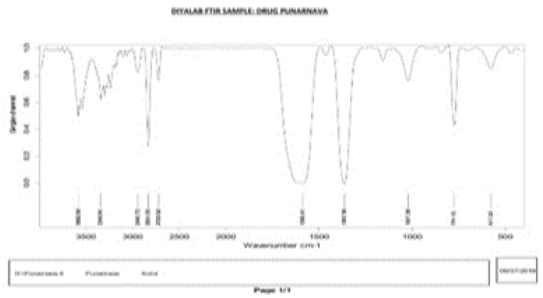
Aggregation assay was performed as per method previously described by Hess et al. [2000] with minor modifications. Briefly, freshly prepared solution of 10 mM calcium chloride di-hydrate and 1.0 mM sodium oxalate, containing 200 mM NaCl and 10 mM sodium acetate trihydrate, was adjusted to pH 5.7 .All experiments were performed at 37 °C, using a circulating water bath. For crystallization experiments, 25 ml of sodium oxalate solution was transferred into a beaker and placed in the hot plate magnetic stirrer, which was maintained at 37 °C and constantly stirred at 800 rpm. An additional 1 ml of distilled water/standard (cystone) /extract were added and finally calcium chloride solution (25 ml) was added. The optical density was measured at 620 nm in spectrophotometer (UV 1800, Shimadzu Corporation, Japan) after addition of calcium containing solution, on every 15 s over 5 min and then every 1 min over 10 min. Percent inhibition in the presence of cystone was compared with the control by the following formula. The percentage inhibition was calculated as: [1-(Tsi/Tsc)]*100, where Tsc, the turbidity slope of the control; and Tsi, the turbidity slope in the presence of the inhibitor.

Formulation :

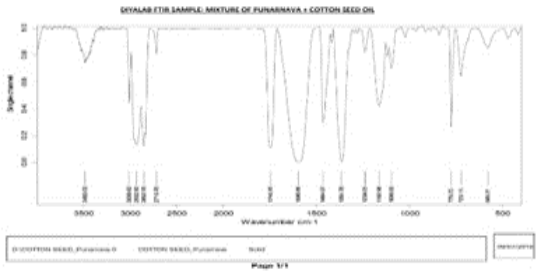
IV RESULTS & DISCUSSION

Compatibility studies : (A)





(B)



(A+B)

A, B, A+B: are FTIR spectra of Punarnava, Cottonseed oil & Mixture of both.

The FTIR proves compatibility of punarnava & cotton seed oil with no characteristic changes.

IR Spectrum	Groups	Peaks (CM-1)
Boerhavia diffusa Linn	-OH-(Strong) -CH- -NH- C=O	3582.58 2940.72 2720.52 1586.41
Cotton seed oil	-OH- -OH- -C-OR- -C=O -NH-	3471.46 3008.58 1747.90 1588.09 2716.89
Mixture of Boerhavia diffusa+ Cotton seed oil.	-OH-(Strong) -CH- -NH- -C-OR--C=O	3483.63 2932.80 2716.76 1748.78 1598.98

DSC Spectras :

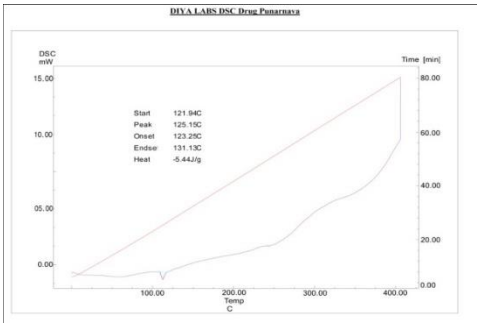


Fig : C

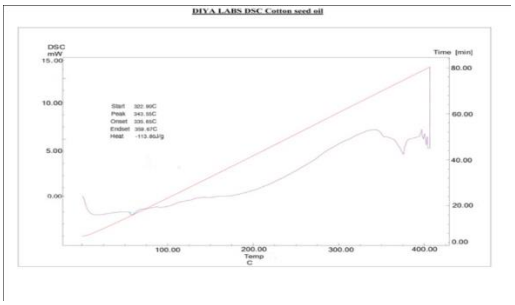


Fig : D

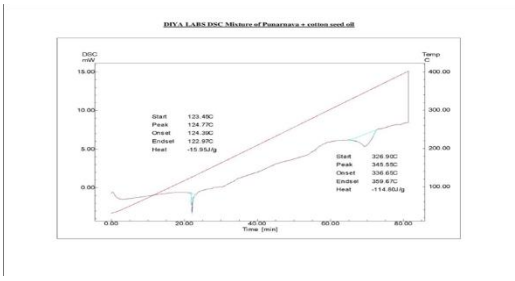


Fig : (C+ D)

C, D Shows DSC Spectra at 125.15° C, 343.55° C resp of Drug & Oil.

C+D Spectra of mixture shows Compatibility with eachother.

In vitro Anti- urolithiasis activity :

A : Nucleation assay :-

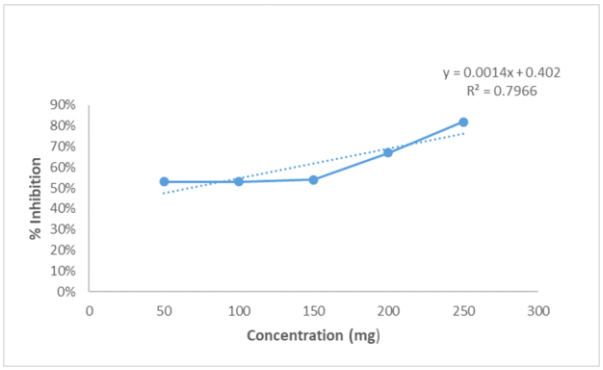


Fig:1 : Percent inhibition of Punarnava (ethanolic extract)

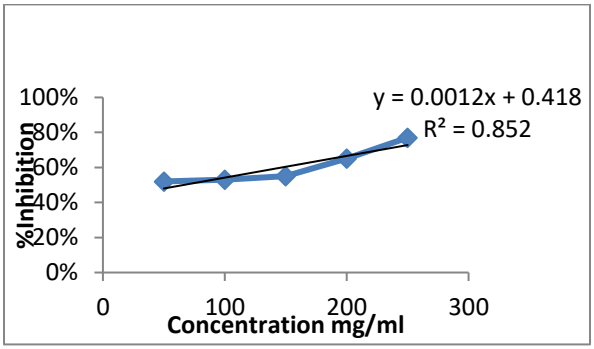


Fig 2 Percent inhibition of Punarnara (aqueous extract).

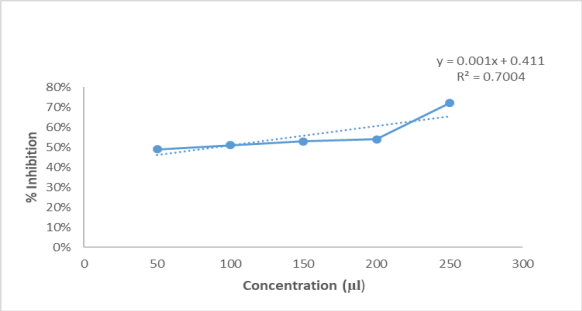


Fig 3 Percent Inhibition of cotton seed oil.

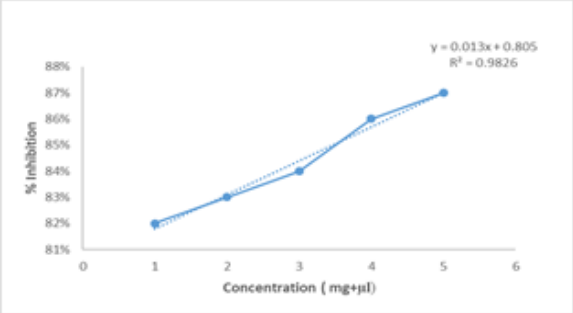


Fig 4 : Percent inhibition by mixture (Punarnava and cotton seed oil)

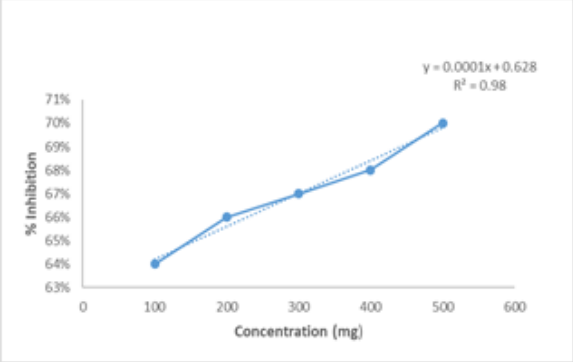


Fig 5 : Percent inhibition of control (Cystone tablet)

Stone formation begins with the onset of tiny crystals in the kidney. Crystals are composed of substances such as calcium and oxalate that have been filtered into the urine. These salts bind to form the central core called the nuclei. The combination of solution of calcium chloride and sodium oxalate resulted in the formation of calcium oxalate crystals. The rate of nucleation was estimated by the comparison of induction time in the presence of the extract with that of control. The absorbance was measured at 620nm. An increase in crystal dissolution decreased the turbidity of solution. The data represents that the percent inhibition of crystals formation was directly proportional to increase in concentration of plant extract, with maximum activity observed at 250mg Punarnava+ 250 µl Cottonseed oil. Between two different extracts studied, the ethanoic extract of *Boerrhavia diffusa* along with cottonseed oil was able to render maximum inhibition against CaOx crystallization on comparison with aqueous extract of *Boerrhavia diffusa* as observed in results. The result of the nucleation assay indicate that the interference of the extracts with the crystal formation may be a therapeutic strategy to hamper stone formation. This ability of extract to reduce the nucleation and increase the oxalate excretion in urine, and prevent the precipitation of the CaOx crystals, indicate the presence of inhibitory compound in the extracts.

B: Aggregation Assay :-

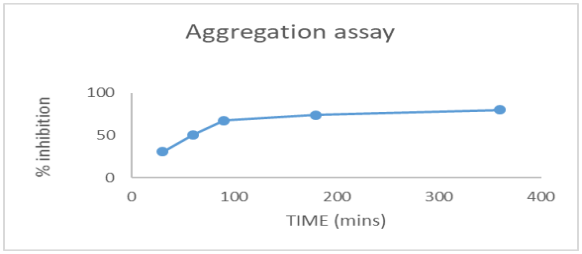


Fig : 6

Aggregation is the clumping of small particles into larger ones. In this process, crystal solution adhere together and form large particles. In various steps of stone formation, crystal aggregation is more important step rather than nucleation because aggregation occurs within a few seconds**.** The result of aggregation assay showed a similar trend to that of the nucleastion assay. Crystals treated the combination of drugs were less aggregated, while the rate of inhibition elevated with increase in concentration of the extract of punarnava and cotton seed oil.[33]

Evaluation of Tablets:-

The hardness, thickness an friability of tablet were found to be 4.25 Kg/sq.cm , 3.52 mm and 0.9% respectively and the dispersion time of tablet was found to be 3 min.

VI. CONCLUSION

A combination of drug is a medicine which includes two or more active ingredients combined in single dosage form. Boerhavia diffusa L. commonly known as Punarnava is phytochemically rich in steroids, alkaloids, glycosides, flavonoids and phenols. Traditionally the plant is used in treatment of Anti

diabetic, Hepatoprotective, immunomodulatory, Analgesic activity etc. Since the plant is used traditionally as Hepatoprotective it is worthwhile to evaluate its anti-urolithiatic activity. Cotton seed oil is basically used as a food ingredient. It have emollient properties and also help in maintaining or regulate blood pressure. Thus can be consumed by patients suffering from heart disease. In the reported study of cotton leaf extract it has been said that urolithiatic activity can be achieved. Hence the other part of cotton plant i.e. cotton seed was evaluated for anti urolithiatic analysis. Using dried ethanolic extract of Punarnava and cottonseed oil, phytochemical characterization, U.V. Spectrophotometric determination, in-vitro anti urolithiatic analysis was carried out. This study was aimed to evaluate the anti urolithiatic activity of combination used by in-vitro method. The activity was compared to standard dosage form Cystone. Cystone is a marketed dosage form used to treat urolithiasis with multiple drugs incorporated in each tablet. Thereafter a tablet formulation was developed consisting of Punarnava, cottonseed oil, and ginger powder. Ginger powder acts as an antiemetic. As in urolithiasis emesis is the commonly encountered effect due to nausea which propels out the administered drug. Incorporation of ginger powder in tablet formulation was aimed to overcome the drawback and improve the residence time of dosage form in body. The antiurolithiais activity was carried out by in-vitro Nucleation and Aggregation assays (i.e. artificial urine preparation/ crystal formation). The ethanolic extract of roots of Punarnava and cottonseed oil (250mg+250 µl) was found to be effective at greater extent. Hence the formulation was prepared with the effective concentration of the combination of drug. After completion of the experimental work we are at the conclusion that ethanolic Punarnava root extract along with cottonseed oil can be used readily for preparation and characterization of new drug delivery system.

REFERENCES

[1] 1.HarshikaAwasthi, Dayanandan Mani, RajendraNath, AnuradhaNischal, KauserUsman, Sanjay Khattri, "Standardization, Preparation And Evaluation Of An *Ayurvedic*Polyherbal Formulation In Capsule Dosage Form Suitable For Use In Clinical Trials", IAJPR-2014

[2] 2.WHO traditional medicine strategy, *WHO Library Cataloguing-in-Publication Data* 2014-2023; pg no. 5-70.

[3] 3. Brahmbha A, Seth AK, Jammula SB, et al, Formulation and evaluation of taste masked oral disintegrating tablet of ondesarton hydrochloride. Indo American Journal of pharmaceutical Research 2011, 2, 91-99.

[4] N P S Sengar, RiteshAgrawal, AshwiniSing,A textbook of pharmacognosy, ,2009, pg. no. 103

[5] UthayaJayaraman, AnnaduraiGurusamy, "Review on Uro-Lithiasis Pathophysiology and Aesculapian Discussion", *IOSR Journal Of Pharmacy*, 2018; 8, pg no. 30-42.

[6] T. Vijaya, M. Sathish Kumar, N. V. Ramarao, A. NaredraBabu, N. Ramarao, "Urolithiasis and Its Causes- Short Review", *ISSN*, 2013; 2(3): pg no. 1-6.

[7] Jyothi M Joy, S Prathyusha, S Mohanalakshmi, AVS Praveen Kumar, CK Ashok Kumar, "Potent Herbal Wealth WithLitholytic Activity: A Review", *ISSN*, 2012; pg no. 66-75.

[8] A. Skolarikos, M. Strau, T. Knoll, K. Sarica, C. Seitz, A. Petrık, C. Turk, "Metabolic Evaluation and Recurrence Prevention for Urinary Stone Patients: EAU Guidelines", *eur ass urology* ,2014; pg no. 750-763.

[9] C. Barbas, A. Garcıa, L. Saavedra, M. Muros, "Urinary Analysis of Nephrolithiasis Markers", *Journal of Chromatography B*, 2002; (781), pg. no.433-455.

[10] S.R.Radha, Dr.B.Vijayakumari, "Herbal Plants Used In The Treatment Of Urolithiasis", *IJPRD*, 2013; 5(02), pg. no. 66-70.

[11] Rohit Kumar Bijauliya, ShashiAlok, S. K. Jain, V.K. Singh, Devender Singh and Man Singh, "Herbal And Allopathic Medicine For Kidney, Gallbladder And Urinary Stones: A Review", *IJPSR*, 2017; 8(5), pg no. 1935-52.

[12] A. Tiwari, V. Soni, V. Londhe, A. Bhandarkar, D. Bandawane, S Nipate,"An Overview On Potent Indigenous Herbs For Urinary Tract Infirmity: Urolithiasis", *Asian J Pharm Clin Res*, 2012,5(1), pg. no. 7-12.

[13] ShwetaBawari, Archana N. Sah* and D. Tewari, **"**Urolithiasis: An Update on Diagnostic Modalities and Treatment Protocols*",IJPS,*2017; 79(2), pg. no.164-174.

[14] TusharShelke, SandeshWayal, Shiv Gunjegaokar, Sachin Gaikwad, AmolShirsath, Sunil Hadke, "An Overview on Indian Medicinal Plants with Antiurolithiatic Activity", *PRCP*, July-Sept 2014; 4(3): pg no.33-40.

[15] Ahmed Bensatal Æ M. R. Ouahrani, "Inhibition of crystallization of calcium oxalate by the extraction of Tamarixgallica L", *IJPSR*, 2008; 36, pg no. 283–287.

[16] Rathod N, ChitmeHr, Chandra R, "*In Vivo And In Vitro* Models For Evaluating Anti-Urolithiasis Activity Of Herbal Drugs", IJPRBS, 2014;3(5), pg. no. 309-329.

[17] R.R. Bhagwat and I.S. Vaidhya, "*Novel Drug Delivery Systems: An Overview*", IJPSR (2013), 4(4).

[18] PranatiNayak, M Thirunavoukkarasu, "*A review of the plant Boerhaaviadiffusa: its chemistry, pharmacology and therapeutical potential",* JPHYTO, 2016; 5(2), pg. no. 83-92.

[19] ManjushaChoudhary, Vipin Kumar, Hitesh Malhotra,Surender Singh, Medicinal plants with potential antiarthriticactivity, JIE , Apr-Jun 2015 ,Vol 4 ,Issue 2, pg. no. 147.

[20] N. K. Jain, Controlled and Novel drug delivery, first edt, 2019,pg. no. 1-2.

[21] Dr. Dheeraj T. Baviskar et. al. Novel drug delivery systems, third edt, sep 2016, pg. no. 2.1 .

[22] Joseph R. Robinson et. al. Controlled Drug Delivery Fundamentals and Applications, second edt, vol. 29, pg. no. 9-11.

[23] Ali Nokhodchi, Shaista Raja, Pryia Patel, Kofi Asare-Addo, The Role of Oral Controlled Release Matrix Tablets in Drug Delivery Systems,BioImpacts, 2012, 2(4),pg. no. 175-187.

[24] SathishUmmadi, B. Shravani, N. G. RaghavendraRao, M. Srikanth Reddy, B. SanjeevNayak, Overview on Controlled Release Dosage Form, International Journal of Pharma Sciences, Vol. 3, No. 4 (2013), pg. no. 258-269.

[25] DebjitBhowmik, Harish Gopinath, B. Pragati Kumar, S. Duraivel, K. P. Sampath Kumar, Controlled Release Drug Delivery Systems, The Pharma Innovation, Vol. 1 No. 10 2012, pg. no. 24.

[26] Dinesh K. Agarwal ,Phundan Singh , MuktaChakrabarty, A J Shaikh, S G Gayal, Cottonseed Oil Quality, Utilization And Processing, Technical Bulletin from Central Institute for Cotton Research Nagpur, NO: 25 , pg. no. 3 –4.

[27] Ibanga O. Isaac, Okon D. Ekpa, Fatty Acid Composition of Cottonseed Oil and its Application in Production and Evaluation of Biopolymers,American Journal of Polymer Science 2013, 3(2): 13-22.

[28] Natalia Davydova, Erika Stippler, Ping Jin, Gabriel Giancaspro, Development and validation of a dissolution test method for vitamin A in dietary supplement tablets, Journal of Pharmaceutical and Biomedical Analysis 53 (2010), pg. no. 295- 301.

[29] D. Monterroza, L. Ponce De Leon, Development of a USP Apparatus 3 dissolution method for progesterone soft gelatin capsule, Softi gel, pg. no. 1-10.

[30] J. Shakina , A. Muthuvinothini, Synthesis and Characterization of Cotton Seed Oil based Biodegradable Thermosetting Polymers, vol 3, March 2015, pg. no. 526.

[31] Reff for nucleation assay

[32] DeeptiSharmaa, YaduNandanDeya, InduSikarwarb, RichaSijoriaa, Manish M. Wanjaria,*, Ankush D. Jadhava, In vitro study of aqueous leaf extract ofChenopodium album for inhibition of calcium oxalate and brushite crystallization, egypt i a n j o u r n a l o f b a s i c a n d a p p l i e d s c i e n c e s 3 (2 0 1 6) 1 6 4 – 1 7 1.

[33] Ratkalkar and Kleinman 2011.