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In vitro growth inhibition study of hydroxyapatite crystals in the presence of selected herbal extract solutions

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ABSTRACT

Hydroxyapatite, is the most stable mineral found in body. Hydroxyapatite crystals were grown by the gel technique in the form of periodic Liesegang ring patterns. The size of fine crystalline hydroxyapatite was found in order of microns. The harvested crystals were characterized by Powder X- Ray Diffraction (XRD). Aqueous, alcoholic, hydro-alcoholic extracts of five herbs selected based on Reverse Pharmacology (RP) experiential data of Ayurveda were used for the growth inhibition study of hydroxyapatite crystallites. These extracts were compared with $CaCl_2$ (control) solution to study their effects in terms of the particle size of crystallites, the total diffusion length in the gel column, the number of rings and the spacing between two rings. The inhibition in hydroxyapatite crystal growth by the herbal extracts is reported.

Key words: Hydroxyapatite; Gel growth; Herbal extracts; Inhibition; Reverse Pharmacology

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INTRODUCTION

There are numerous technological applications of bio-materials and bio-ceramics. One of the most important objectives is to develop a new material for bone substitution. The diversity of the precise chemical composition of various calcium phosphates is roughly equivalent to that of the inorganic matrix of the human bone and they are found to be the most suitable as implant materials [1]. The major phase found in bone is hydroxyapatite, or Basic Calcium Phosphate (BCP), $C_{10}(PO_4)_6(OH)_2$, and the other commonly known phases are octa-calcium phosphate , tri-calcium phosphate, di-calcium phosphate di-hydrate and di-calcium phosphate [2]. Hydroxyapatite is not only bio-compatible but is also a bioactive material; it directly bonds to the bone and favors the implant fixation due to its various interesting properties like, osteo-conductivity, tissue compatibility and non-antigenic nature [3,4]. Hydroxyapatite has been used to study the hard tissue calcifications such as bone and teeth and also the undesirable pathological mineralization of the articular cartilage [5,6], cardiac valves [7,8] and kidney stones [9]. Apart from these, hydroxyapatite has several other diverse applications, for example, in catalysis [10], in drug delivery system [11], in fuel cells [12] and in gas sensing [13]. The apatite crystal deposition induced inflammation is a common finding in bursitis and peri-arthritis, where the most joints or bursae can be involved, with more common sites

including shoulders, hips, knees and digits. Apatite can also be associated with scleroderma and other connective tissue diseases like osteoarthritis and erosive arthritis [14].

It is worth noting that different techniques have been employed by various workers to grow hydroxyapatite crystals, viz., precipitation from aqueous solution [15,16], hydrothermal method [17], flux growth [18] and high temperature sintering [19]. Also, the attempts have been made to obtain hydroxyapatite bio-ceramics by wet chemical process [20] and microwave irradiation [13]. Except the reported work of Ashok *et al.* [3] and Parekh *et al* [21], notwithstanding, no major study is reported on the gel growth of hydroxyapatite crystals. Earlier this gel based *in vitro* model was used to mimic the physiological condition and was helpful to identify the potent herbal extracts based as well as certain fruit juice based inhibitors of various biomaterials crystals successfully, such as calcium oxalate [22], monosodium urate monohydrate [23], brushite [24], struvite [25] and hydroxyapatite [21]. However, it is interesting to note that the herbal extracts based inhibitors are mainly studied so far for the adherence of bacterial colonies on saliva coated hydroxyapatite for dental plaques, for example, herbal extracts of *Andrographis paniculata*, *Cassia alata*, *Camellia sinensis*, *Psidium guajava, Harrisonia perforata* and *Streblus asper* [26]; seaweed extracts [27] and *Azadirachta indica* extracts [28].

The present investigation deals with the growth inhibition studies of gel grown hydroxyapatite crystals. This study is important because it uses a simple gel based model for the inhibition of hydroxyapatite under the normal growth condition by the herbal extracts. Hydroxyapatite crystals are grown in the silica gel media in the form of Liesegang rings. The effect of five selected herbal extract solutions, viz. *Boswellia serrata* Roxb. (Shallaki) gum resin, *Tribulus terrestris* Linn. (Gokshura) fruit, *Rotula aquatica* Lour. (Pashanbheda) root, *Boerhaavia diffusa* Linn (Punarnava) root and *Commiphora wightii* Engl. (Guggul) resin, on the Liesegang ring formation is studied. As the Liesegang rings are formed of hydroxyapatite crystals, the effect of herbal extracts on the Liesegang rings brings the knowledge of growth and inhibition of crystals. This present investigation forms an exploratory intervention of reverse pharmacology.

MATERIALS AND METHODS

2.1 Crystal Growth

Glass test tubes of 25 mm diameter and 150 mm length were selected for the growth of crystals. Sodium metasilicate solution of specific gravity 1.06 was acidified by 1N ortho phopheric acid so that the pH of the mixture could be set within 6.0 to 6.5. This mixture was transferred into different test tubes and allowed to set into the gel form. After setting the gel, 1M calcium chloride solution was poured gently on the set gel. Good quality very small hydroxyapatite crystals were grown in the form of Liesegang rings as shown in Figure 1.

Crystal size was determined by Master Sizer 2000. Powder X-Ray Diffraction (XRD) of the samples was carried out using PW1710 BASED system with Cu-K_α radiation. The powder XRD data were analyzed using the software powder-X.

2.2 Selected Plant Extracts:

Five selected plants with Ayurvedic usage, viz., *Boswellia serrata* Roxb. (Sallaki) gum resin, *Tribulus terrestris* Linn. (Gokshura) fruit, *Rotula aquatica* Lour. (Pashanbheda) root, *Boerhaavia diffusa* Linn (Punarnava) root and *Commiphora wightii* Engl. (Guggul) resin were extracted with alcohol, 50% hydro-alcohol by soxhelet method of extraction using 50g of plant material. Hot aqueous extracts were prepared by boiling 50g plant material in 400ml of water reducing to half the volume.

For growth inhibition studies the 1 % of selected herbal extracts of *Boswellia serrata* (Sallaki) gum resin, *Tribulus terrestris* (Gokshura) fruit, *Rotula aquatica* (Pashanbheda) root, *Boerhaavia diffusa* (Punarnava) root and *Commiphora wightii* (Gugull) resin were added along with calcium chloride control solution as the supernatant solution to be poured on the set gels. All glass wares were autoclaved before use. The laminar flow hood was used to avoid microbial contamination.

RESULTS AND DISCUSSION

Since hydroxyapatite is the most stable calcium phosphate salt under physiological conditions, the gel grown crystals were characterized by particle size analysis and powder XRD. The crystals were in form of small crystallites in the periodic formation of Liesegang rings as shown in Figure 1.

Figure 1 : Hydroxyapatite (HA) crystal growth in the form of Liesegang rings

Table 1 shows the particle size measurement of hydroxyapatite crystals obtained from aqueous, ethanolic and hydro-ethanolic extracts. From this table it can be interpreted that 10% of the particles (or crystallites) of hydroxyapatite were less than 47.25 micron, 43.64 micron and 46.18 micron for aqueous, ethanolic and hydro-ethanolic extracts, respectively. While 50% of the particles were less than 82.68 micron, 77.01 micron and 46.18 micron for aqueous, ethanolic and hydro-ethanolic extracts, respectively. Ninety percent of the particles were less than 107.54 micron, 132.08 micron and 135.46 micron for aqueous, ethanolic and hydro-ethanolic extracts, respectively. This closely corresponds to our earlier results [21].

Table 1: Particle size measurement of HA crystals obtained from aqueous, ethanic and hydro-ethanolic extracts. (HA -Hydroxyapatite, d – Diameter)

Sample code	d (0.1) micron	d (0.5) micron	d (0.9) micron
HA (Aqueous)	47.25	82.68	107.54
HA (Ethanolic)	43.64	77.01	132.08
HA (Hydroethanolic)	46.18	79.47	135.46

It was observed that the minimum particle size was found in the case of aqueous extracts, which was followed by ethanolic and hydroethanolic extracts. Looking at table 1 for 90% particles size distribution the aqueous extracts show the minimum size followed by ethanolic and hydroethanolic. This study corresponds to our earlier studies on hydroxyapatite in which we studied the growth inhibition in terms of diffusion constant measurements in the presence of herbal extracts [21].

Figure 2 exhibits the powder XRD pattern of hydroxyapatite crystals. The crystal structure was found to be monoclinic with unit cell parameters as, a =9.432 Å, b=18.843 Å, c =6.923 Å, $\alpha = \beta = 90.01^0$, $\gamma = 119.05^0$. The values are closely matching with those reported by Elliot *et al*. [29].

Acute, sub-acute and chronic musculoskeletal symptoms, such as bursitis and tendonitis, are also known to occur in association with calcium apatite deposition at joints other than shoulders [30, 31]. Therefore, many researchers have studied the growth inhibition of hydroxyapatite [32-35]. Koutsopoulos *et al*. [36-39] have studied the growth and inhibition of hydroxyapatite by different therapeutic drugs and various amino acids [40-43]. Pampena *et al.* [44] have reported the inhibition of hydroxyapatite by osteopontin phosphopeptides (OPN).

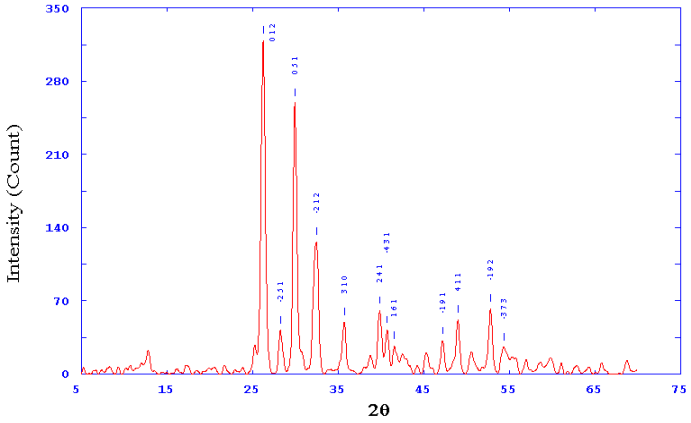


Figure 2: Powder X-ray diffractogram of HA crystals

Hydroxyapatite crystals, in the present investigation, are formed in periodic Liesegang ring structures. Several authors have proposed various theories of Liesegang rings and reported in several systems [45-51]. Among these theories, Henisch and Garcia-Ruiz [45] suggested a numerical method to obtain the formation of Liesegang rings on the basis of Fick's diffusion equation for reagents in gel as a function of time; in another theory, the same authors [46] extended the conditions under which two diffusing reagents could form precipitates either continuous or periodic fashion in a gel media. After forming the position of the first precipitates, the local solute concentrations decreased and as a result further interaction also reduced, after which the interactions could only resume until a second point was found at which the precipitation conditions were again satisfied. A precipitate once formed was assumed to grow until the next precipitation occurred. The precipitates had positions and mass but no explicit thickness; where and when a precipitate would form, this was the outcome of a complex interplay of processes, which depended not only on local concentrations but on the steepness of the supersaturating front and the speed of its advance. However, Chervanskii *et al* [48] took into account the effects of super-saturation, competition between nucleation and droplet growth kinetics as well as redistribution of matter between particles of precipitants.

Small hydroxyapatite crystals were harvested from the Liesegang rings, which were formed due to diffusion of reactants in the gel and the periodic precipitation; therefore, any change in the periodic precipitation or diffusion process, it would invite changes in the Liesegang ring patterns. The effects of the herbal extract solutions on hydroxyapatite crystals were noticed in terms of changes in the Liesegang ring patterns. The growth inhibition study was carried out by considering certain parameters, for instance, the total diffusion length (*D*), the number of Liesegang rings obtained (*N*) and the distance between the two rings (*d*). These distances *D* and *d* were measured by traveling microscope of least count of measurement 0.001cm. The total diffusion length is defined as the distance of the last Liesegang ring from the liquid-gel interface. The 1 % extract solutions were prepared by adding 10 mg concentrated extracts into 10 ml distilled water. The selected plants for extraction were *Boswellia serrata* gum resin, *Tribulus terrestris* fruit, *Rotula aquatica* root, *Boerhaavia diffusa* root, and *Commiphora wightii* resin. The plants were identified by taxonomists; their phytochemical properties were studied and reported elsewhere along with the details of herbal extract preparations [52].

From the experiment it was observed that the pure calcium chloride supernatant solution (control) gave thick, sharp rings with deep diffusion in the gel with comparison to other extracts. In the case of *B. serrata*, the thick, sharp rings having comparatively less in numbers and comparatively less total diffusion length in the gel were noticed, while *T. terrestris* gave spiral rings, which were interconnected with each other and the total diffusion length was the same as that of pure $CaCl_2$. For *R. Aquatica*, the rings were sharp and very thin but the number of rings was more and the total diffusion length was comparatively less. However, for *B. diffusa*, the number of rings was the same but the last few rings were not completely formed and the total diffusion length was also the same. The same types of results were observed in the case of *C.wightii*. The experiments were carried out in the aseptic medium in laminar flow hood to avoid fungal growth and repeated several times. Table 2 compiles the results in terms of number of Liesegang rings and the total diffusion length. When the total diffusion length *D* is small, it indicates the less amount

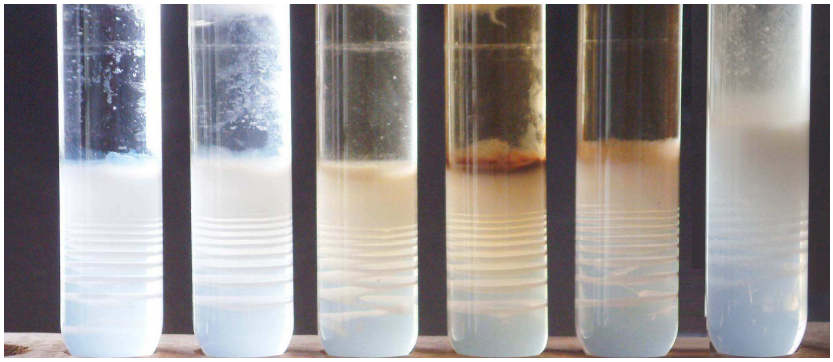
of diffusion of supernatant solution in to the gel. If the number of rings is less, it also indicates the less periodic precipitation and inhibition of diffusing reactants for the formation of hydroxyapatite.

Table 2: Comparative growth inhibition study of HA in the presence of different herbal extracts

Herbal Extracts	Number of Liesegang Ring	Diffusion length (in cm)
Pure $CaCl_2$	16	3.8
T. terrestris	Spiral ring	3.75
B. serrata	12	2.8
R. aquatica	10	2.75
B. diffusa	12**	2.75
C.wightti	12**	2.75

** *Last two rings are completely not formed*

Figure 3 : Comparison of growth of HA crystals in the form of Liesegang rings in different herbal extract solution; pure calcium chloride, *B. serreta;* T. *terrestris ; R. aquatica; B. diffusa and C. wightti* (from left to right)



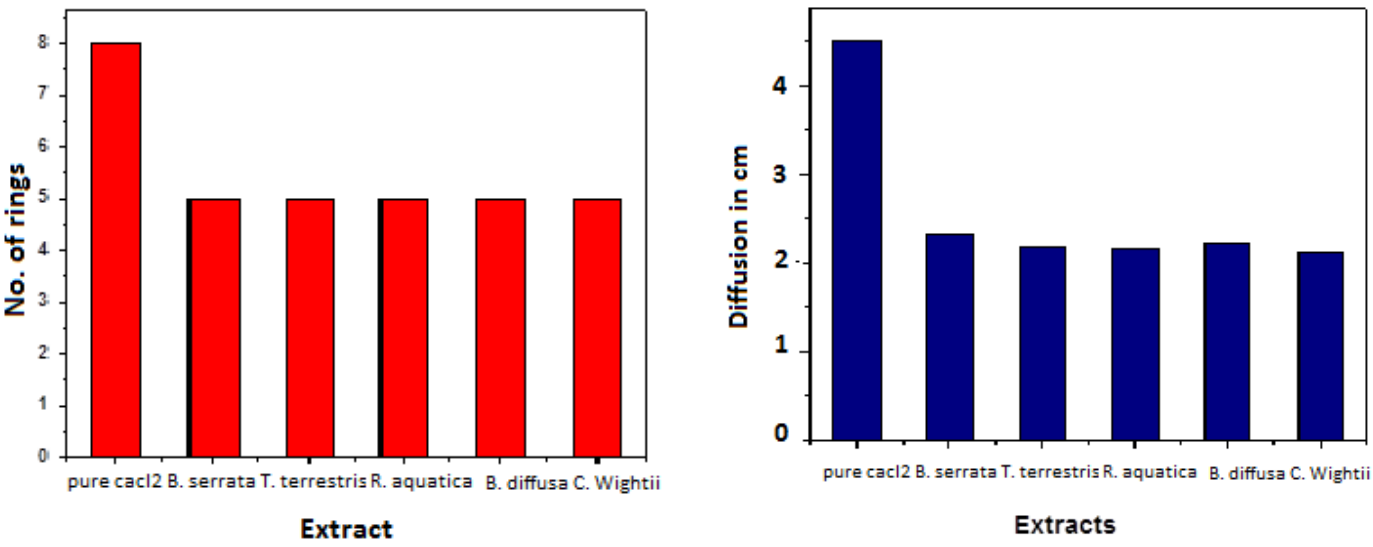


Figure (4a and b): Histograms of number of rings and diffusion distance in gel for different herbal extracts

The average thickness of Liesegang rings was 0.09 to 0.1 cm in pure $CaCl_2$- control solution; except for *R. aquatica*, it was of the order of 0.079 cm. It is concluded that *R. aquatica*, exhibits comparatively more inhibition of hydroxyapatite precipitation. The total diffusion length and the number of rings are comparatively less in *R. aquatica.* Figure 3 is a photograph showing test tubes with different herbal extract solutions. Figure 4 a, b exhibits the histograms of the number of Liesegang rings *N* and diffusion length *D*, respectively, in the presence of various

herbal extracts and pure $CaCl_2$ solution. From Figure 4 a, b it is clearly indicated that the reduction in the number of Liesegang rings as well as the reduction in the total diffusion length occur in the presence of different herbal extracts.

Figure 5 shows a set of plots drawn for the spacing between two consecutive rings *d* versus the number of rings *N*. From these plots it can be clearly seen that *R. aquatica* gives maximum spacing between the initial two rings and then it increases exponentially, which indicates that it delayed the periodic precipitations in the gel. On the other hand, all other extracts do not exhibit significant change in the ring spacing indicating that they do not bring significant change in the periodic precipitation. However, the *B. diffusa* extract did not bring large change in the periodic precipitation but brought better changes in the diffusion length. Similar results were observed for *B. serrata*. Single factor ANOVA statistical analysis was applied to the plots of Figure 5, which shows that the behaviors are highly significant ($P< 0.0001$).

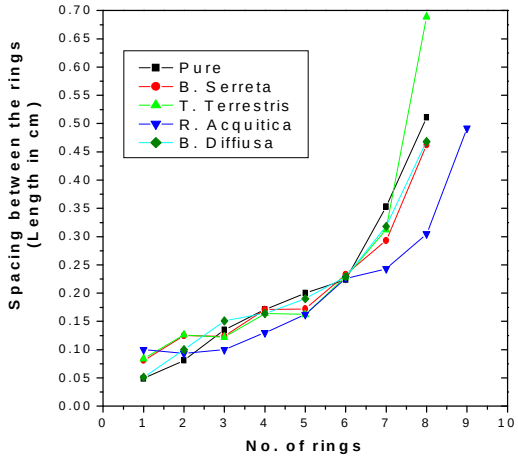


Figure 5: The plots of spacing between two consecutive rings versus the number of rings

Also, the ethanlolic as well as the hydro-ethanolic extracts of the same plants were used. In case of ethanolic extracts, the number of rings was reduced. All extracts had the same number of five rings and the first band thickness was 2.223 cm. The total diffusion length in the gel was 4 cm for *B. serrata*, *R. aquatica* and *C. wightii*; whereas, in the case of *B. diffusa* and *T. terrstris* it was 3.5 cm. For hydro-ethanolic extracts, thick bands were observed for all extracts and it was not possible to measure the spacing and other parameters. The width of band was in average 4.3 cm for all extracts.

The gel growth of hydroxyapatite at physiological temperature is important for growth inhibition studies. This serves as a simple screening model for identifying the potent inhibitors of hydroxyapatite for therapeutic purposes. In the study, *R. aquatica* and *B. diffusa* extracts have been found to be good inhibitors of hydroxyapatite *in vitro*. These extracts were also found to be good inhibitors *in vivo* and also through animal model studies, which are reported elsewhere [52]. These herbal extracts are extensively studied for alternate medicine, i.e., Ayurveda, point of view, which may also prove to be useful in the mode of reverse pharmacology. Reverse pharmacology, a trans-discipline, which initiates drug discovery and development from the traditional knowledge and practice through robust and objective documentation. It also systematically explores Ayurvedic and current scientific mechanisms, as to satisfy the safety, efficacy and therapeutic conditions for developing either an extract or a molecule as a new drug [53-56].

CONCLUSION

Hydroxyapatite crystals were grown in the form of Liesegang rings in silica hydro gel, the crystals size was in order of microns. Powder XRD confirmed the monoclinic crystal structure, and the unit cell values correspond with the earlier reported values. Pure calcium chloride containing supernatant solution served as a control solution for growth inhibition study of hydroxyapatite crystals. Out of five selected herbal extracts, the aqueous extracts of *R. aquatica*

and *B. diffusa* have demonstrated good results in terms of periodic precipitation and less total diffusion length, respectively. Ethanolic and hydroethanolic extracts did not show good inhibition in comparison to aqueous extracts. The gel based *in vitro* growth inhibition study of hydroxyapatite was carried out at physiological temperatures and could be useful for screening the potent inhibitors of hydroxyapatite in physiological conditions.

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REFERENCES

[1] J. C Merry, I. R. Gibson, S. M. Best, W. Bonfield, *J. Mater. Sci. Mater. Med.*, **1998**, 9,779.
[2] R. Z. LeGeros, *Chem. Rev.*, **2008**,108,4742.
[3] M. Ashok, N. M. Sundaram, S. N. Kalkura,. *Mater. Lett*., **2003**, 57, 2066.
[4] R. Z. LeGeros, J. P. LeGeros, In: L. L. Hunch, J. Wilson (Ed.), Dense Hydroxyapatite. In An Introduction to Bio-ceramics (World Scientific, Singapore, **1993**).
[5] G. V.Gordon, T. Villanueva, H. R. Shumacher, V. J Gohel, *J. Rheumatol.*, **1984**, 11, 68.
[6] A. L. Boskey, P. G. Bullogh, *Scan. Electron Microscop*., **1985**, 28, 511.
[7] F. J. Schöen, R. J. Levy, *Ann. Throac. Surg.*, **2005**, 79, 1072.
[8] M. Valente, U. Bortloti, G. Thiene, *Am. J. Pathol.*, **1985**, 119, 12.
[9] G. H. Nancollas, *J. Cryst. Growth.*, **1974**, 42, 185.
[10] P. A. Kumar, M. P. Reddy, L. K. Ju, H. H. Phil, *Catal. Lett*., **2008**, 126, 78.
[11] R. N. Panda, F. H. Ming, R. J. Chung, T. S.Chin, *Jpn. J. Appl. Phys*., **2001**, 40, 5030.
[12] M. A. Varghese, C. F. Gonzalez, M. M. Gallego, J. D. Solier, I. Cachadita, E. A. Matijevic, *J. Mater. Res*., **2000**, 15, 33.
[13] M. P. Mahabole, R. C. Aiyer, C. V. Ramakrishna, B. Sreedhar, R. S. Khairnar. *Bull. Mater. Sci*., **2005**, 28, 53.
[14] P. A. Dieppe, M. Doherty, D. G. MacForlane, C. W. Hutten, J. W. Bradfield, I. Watt, *Rheumatol*., **1984**, 23, 84.
[15] (15) J. Arendes, J. Chirstoffersen, M. R. Chirstoffersen, H. Eckert, B. D. Flower, J. C. Heughebaert, G. H. Nancollas, J. P. Yensinowski, S. J. Zawacki, *J. Cryst. Growth.*, **1987**, 84, 515.
[16] P. W.Brown, N. Hocker, S. Hoyle, *J. Am. Ceram. Soc*., **1991**, 74, 1848.
[17] M. Ashok, S. N. Kalkura, N. M. Sundaram, D. Arivuoli, *J. Mater. Sci. Mater. Med.*, **2007**, 18, 895.
[18] A. C. Tas, *Powder Diffract*., **2001**, 16, 102.
[19] A. C. Tas, F. Korkusuz, M. Timucin, N. Akkas, *J. Mater. Sci: Mater. Med*., **1997**, 8, 91.
[20] N. Özgür Engin, A. C. Tas, *J. Am. Ceram. Soc.*, **2000**, 83, 1581.
[21] B. Parekh, M. Joshi, A. Vaidya, *J. Cryst. Growth*., **2008**, 310, 1749.
[22] V. S. Joshi, B. B. Parekh, M. J. Joshi, A. B. Vaidya, *J. Cryst. Growth*., **2005**, 275:e ,1403.
[23] B. B. Parekh, S. R. Vasant, K. P. Tank, A. Raut, A. D. B. Vaidya, M. J. Joshi, *Am. J. Inf. Dise.*, **2009**, 5, 225.
[24] K. C. Joseph, B. B Parekh, M. J. Joshi, *Curr. Sci.*, **2005**, 88, 1232.
[25] C. K. Chauhan, M. J. Joshi, *Urol.Res.*, **2008**, 36, 265.
[26] J. Limsong, E. Benjavongkulchai, J. Kuvatanasuchati, *J. Ethnopharmacol*., **2004**, 92, 281.
[27] Y. Saeki, *Bull. Tokyo Dent. Coll*. **1994**, 35, 9.
[28] L. E. Woolinsky, S. Mania, S. Nachnani, S. Ling, *J. Dent. Res.*, **1996**, 75, 816.
[29] J. C. Elliot, P. E. Mackie, R. A. Young, *Science.*, **1973**, 180, 1055.
[30] R. J. R. McKendry, H. K. Uhthoff, K. Sarkar, P. S. Hyslop, *J. Rheumatol.*, **1982**, 9, 75.
[31] H. K. Uhthoff, K. Sarkar, J. A. Maynard, *Clin Orthop.*, **1976**, 118, 164.
[32] G. K. Hunter, B. L. Allen, M. D. Grynpas, P. T. Chen, *Biochem. J.*, **1985**, 228, 463.
[33] R. W. Romberg, P. G. Werness, B. L. Riggs, K. G. Mann, *Biochemistry*., **1986**, 25, 1176.
[34] A. L. Boskey, M. Maresca, W. Ullrich, S. B. Doty, W. T. Butler, C. W. Prince, *Bone Miner.*, **1993**, 22, 147.
[35] J. J. Damen, J. M. Ten Cate, *Arch Oral Biol.*, **1994**, 39, 141.
[36] S. Koutsopoulos, J. Demakopoulos, X. Argiriou, E. Dalas, N. Klouras, N. Spanos, *Langmuir.*, **1995**, 11, 1831.
[37] S. Koutsopoulos, E. Dalas, N, Tzavellas, N. Klouras, *J. Chem. Soc. Faraday Trans*., **1997**, 93, 4183.
[38] S. Koutsopoulos, E. Dalas, N. Tzavellas, N. Klouras, P. Amoratis, *J. Cryst. Growth.*, **1998**, 183, 251.

[39] S. Koutsopoulos, E. Pierri, E. Dalas, N. Tzavellas, N. Klouras, *J. Cryst. Growth.*, **2000**, 218, 353.
[40] S. Koutsopoulos, E. Dalas, *Langmuir.*, **2001**, 17, 1074.
[41] S. Koutsopoulos, E. Dalas, *J. Coll. Interface. Sci*.,**2000**, 231, 207.
[42] S. Koutsopoulos, E. Dalas, *Langmuir*., **2000**, 16, 6739.
[43] S. Koutsopoulos, E. Dalas, *J. Cryst. Growth.*, **2000**, 216, 443.
[44] D. A. Pampena, K. A. Robertson, O. Litvinova, G. Lajoie, H. A. Goldberg, *Biochem J.* **2004**, 378, 1083.
[45] H. K. Henisch, J. M. Garcia-Ruiz, *J. Cryst. Growth.*, **1986**, 75, 195.
[46] H. K. Henisch, J. M. Garcia-Ruiz, *J . Cryst. Growth.*, **1986**, 75, 203.
[47] B. Chopard, P. Luthi, M. Droz, *J. Stat. Phys.*, **1994**, 76, 661.
[48] D. S. Chernvskii, A. A. Polezhaev, S. C. Muller, *Physica D.*, **1991**, 54, 160.
[49] Y. Brechet, J. S. Kirkaldy, *J. Chem Phys.*, **1989**, 90, 1499.
[50] G. K. Kirov, *J. Cryst. Growth*., **1972**, 15, 102.
[51] K. C. Joseph, M. J. Joshi., *Ind. J. Phys*., **2002**, 76A, 159.
[52] A. Raut, N. Pandita, M. J. Joshi, S. Mengi. "*Preclinical Development of Herbal Formulation for Inflammatory Arthropathy:* Report submitted to Department of Biotechnology, Govt. of India, New Delhi, **2002-03**
[53] A. D. B. Vaidya., Reverse Pharmacology- a Paradigm Shift for new drug discovery based on Ayurvedic epistemology, Ayurveda in Transitions, Arya Vaidya Sala, Kottakal, **2010**, 27.
[54] A. D. B. Vaidya, *Ind. J. Pharmacol*., **2006**, 38, 311.
[55] B. Patwardhan, A. D. B. Vaidya, M. Chorghade, S. P. Joshi, *Curr. Bioactive Comp.*, **2008**, 4, 1.
[56] B. Patwardhan, A. D. B. Vaidya, M. Chorghade, *Curr. Sci.*, **2004**, 86, 789.