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# Quantitative Analysis of Calculi in 20 Cases of Canine Urolithiasis

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**A B S T R A C T**

Calculi analysis to determine the chemical composition is important for treatment and prevention of urolithiasis. Quantitative crystallographic analysis using powdered X-Ray diffraction was used to study calculi of 20 cases of canine urolithiasis surgically operated between December 2017 to July 2018 at the TVCC, Nagpur Veterinary College, Nagpur. Pure phase, double phase and triple phase crystalline compounds were identified. The analysis revealed the presence of pure phases like Struvite (05 cases), Calcium carbonate (01), Calcium oxalate monohydrate (06 cases), Gabazine bromide (01 case) and combinations of Struvite and Benitoite (02 cases), Calcium oxalate monohydrate and Calcium carbonate (02 cases), Calcium carbonate and Graphite and 7- aminoheptan-2-one (02 cases), and Struvite and 1,5-penta decanediol (01 case). Amorphous material was also identified besides the mineral composition and the structural composition of the calculi were identified as orthorhombic, tetragonal and monoclinic crystals in 6 samples each, distorted orthorhombic in 5, distorted tetragonal in 3 and graphanic structure in 2 samples. Thus, the XRD was found to be very a useful method for calculi analysis in canine for advising the diet of dogs for preventing chances of recurrence.

## Introduction

For effective treatment and prevention of urolithiasis, determining the chemical composition of the calculi is important. Qualitative and quantitative crystallographic analysis is useful for studying the chemical nature of uroliths. Quantitative crystallography is useful in detecting calcium oxalate, carbonate apatite, cystine, urate, and mixed uroliths (Bovee and McGuire, 1984). Optical crystallographic examination may not be adequate in determining calculi chemicals like silica, occasionally apatite and oxalate, complex salts of uric acid, and crystallized drug metabolites and residues of various types for which methods like X-ray diffraction, electron microprobe, scanning electron microscopy, infrared spectrophotometry and FTIR can be used to give more accurate urolith composition (Ruby and Ling, 1986). In view of this, the investigation was carried out to analyze the calculi using powdered X-Ray diffraction in 20 cases of canine urolithiasis surgically operated between December 2017 to July 2018 at the TVCC, Nagpur Veterinary College, Nagpur.

## Materials and Methods

The X ray defractometry was employed to know the mineral composition of the total 20 uroliths retrieved from the clinical cases. The XRD scan of the synthesized and subsequently sintered powder were recorded using RigakuMiniflex 600 X Ray Diffractometer with CuKα radiation at 40 kV voltage and 15 mA current supply with K-beta filter, scan speed 10.00 deg/min, step width 0.0200deg, scan range 10,000-80,000 deg. The obtained data were the compared with ICDD (International Committee for Diffraction Data) base.

## Results and Discussion

The characterization of the urolith composition is important for treatment of stone related diseases and also helps in understanding the mechanism of its formation and relation to the diet and other environmental effects on dogs of different breeds and regions. The analysis of uroliths was carried out during the present investigation using powder X-ray diffractometer (XRD). Total 20 samples were analyzed that were retrieved during the course of investigation. For characterization of the composition of the urinary calculi, powder XRD pattern of the samples was recorded. The general information regarding the quantitative analysis of the calculi and its chemical composition is given in Table 1, whereas, the Lattice information is presented in Table 2. Out of total 20 samples 13 calculi revealed 1 phase compound, 5 samples revealed 2 phase crystalline compounds and 2 samples revealed 3 phase compounds in the calculi. The analysis revealed the presence of pure phases like Struvite (05 cases), Calcium carbonate (01), Calcium oxalate monohydrate (06 cases), Gabazine bromide (01 case) and combinations of Struvite and Benitoite (02 cases), Calcium oxalate monohydrate and Calcium carbonate (02 cases), Calcium carbonate and Graphite and 7- aminoheptan-2-one (02 cases), Struvite and 1,5-penta decanediol (01 case). Besides the mineral composition, 15 samples also revealed amorphous material. Out of the total 20 calculi samples 28, compounds were noted and the crystalline structure indicated orthorhombic, tetragonal and monoclinic crystals in 6 samples each, distorted orthorhombic in 5, distorted tetragonal in 3 and graphanic structure in 2 samples.

Bovee and McGuire (1984) observed struvite crystals as the most common calculi as also seen in the present investigation. The researchers also reported calcium oxalate, urate, silicate, cysteine and calcium phosphate calculi in dogs. During the present investigation, Struvite uroliths were observed in 8 cases. The culture tests in these cases indicated presence of microorganisms in 5 cases, thus confirming the earlier findings of Bovee and McGuire (1984).

Osborne *et al.*(1999) evaluated calculi composition by X-ray diffraction method and reported that struvite uroliths were the highest followed by calcium oxalate, purine, cystine, calcium phosphate, silica and mixed urolith were also identified. During the present investigation mixed urolith were observed in 7 samples. Bhatt *et al.* (2016) documented presence of calcium oxalate monohydrate struvite and calcium carbonate calculi, whereas mixed constituents contained calcium oxalate hydrate, struvite, whewellite and weddellite in the urinary calculi. However, Corns (1983) opined that no single method of calculi analysis was ideal and a battery of techniques should be used involving a quantitative test to get best results. According to them XRD helped in differentiating similar components in a single calculi however, it did not always help in identifying the minor components in compound composition of calculi.

**Table.1** Calculi analysis - quantitative analysis results (XRD method): General information

| Sr No | Phase/ compound name | Breed | Formula | Nature of stone | Structure | DB ID Number |
|---|---|---|---|---|---|---|
| **1** | Mg (N $H_4$) (P $O_4$) ($H_2$ O)$_6$ – (Struvite) | Great dane | $H_{13}$ $Mg_1$ $N_1$ $O_{10}$ $P_1$ | 1 Phase Crystalline | Orthorhombic | 2106462 |
| **2** | Ca (C $O_2$)$_2$ (H2 O)$_2$.2 – (Calcium carbonate) | Pug | $C_2$ $H_4$.4 $Ca_1$ $O_6$ 2 | 3 Phase Amorphus + Crystalline | Tertagonal | 2310998 |
| | Graphite | | C | | Graphanic | 9012233 |
| | $C_{11}$ $H_{28}$ $Cl$ N O – (7-aminoheptan-2-one) | | $C_{11}$ $H_{28}$ $Cl$ N O | | Monoclinic | 2004934 |
| **3** | Mg (N $H_4$) (P $O_4$) ($H_2$ O)$_6$ - (struvite) | Labrador retriever | $H_{13}$ $Mg_1$ $N_1$ $O_{10}$ $P_1$ | 2 Phase Crystalline | Orthorhombic | 2106462 |
| | Benitoite | | Ba $O_9$ $Si_3$ Ti | | Distorted Tetragonal | 9015502 |
| **4** | $C_2$ $H_2$ Ca $O_5$ -- (Calcium carbonate) | Lhasa apso | $C_2$ $H_2$ Ca $O_5$ | 1 Phase Crystalline | Monoclinic | 2105964 |
| **5** | Mg (N $H_4$) (P $O_4$) ($H_2$ O)$_6$ - (Struvite) | Pug | $H_{13}$ $Mg_1$ $N_1$ $O_{10}$ $P_1$ | 2 Phase Amorphus | Orthorhombic | 2106462 |
| | 1,15-pentadecanediol | | $C_{15}$ $H_{32}$ $O_2$ | | Tetragonal | 2011100 |
| **6** | Mg (N $H_4$) (P $O_4$) ($H_2$ O)$_6$ - (Struvite) | Labrador retriever | $H_{13}$ $Mg_1$ $N_1$ $O_{10}$ $P_1$ | 1 Phase Crystalline | Orthorhombic | 2106462 |
| **7** | Ca (C $O_2$)$_2$ ($H_2$ O)$_2$.2 – (Calcium carbonate) | Mongrel | $C_2$ $H_4$.4 $Ca_1$ $O_6$ 2 | 1 Phase Crystalline + Amorphous | Tetragonal | 2310998 |
| **8** | MRHBr$H_2$0 – (Gabazine bromide) | Dalmatian | $C_{15}$ $H_{18}$ Br $N_3$ $O_3$ | 1 Phase Crystalline + Amorphous | Distorted orthorhombic | 4101243 |
| **9** | $C_2$ $H_2$ Ca $O_5$ - (Calcium oxalate monohydrate) | Pug | $C_2$ $H_2$ Ca $O_5$ | 2 Phase Crystalline + Few Amorphous | Monoclinic | 2105964 |
| | Ca (C $O_2$)$_2$ ($H_2$ O)$_2$.2 - ( Calcium Carbonate) | | $C_2$ $H_4$.4 $Ca_1$ $O_6$ 2 | | Tetragonal | 2310998 |

**Table.1** (Contd….): Calculi analysis -Quantitative analysis results (XRD method): General information

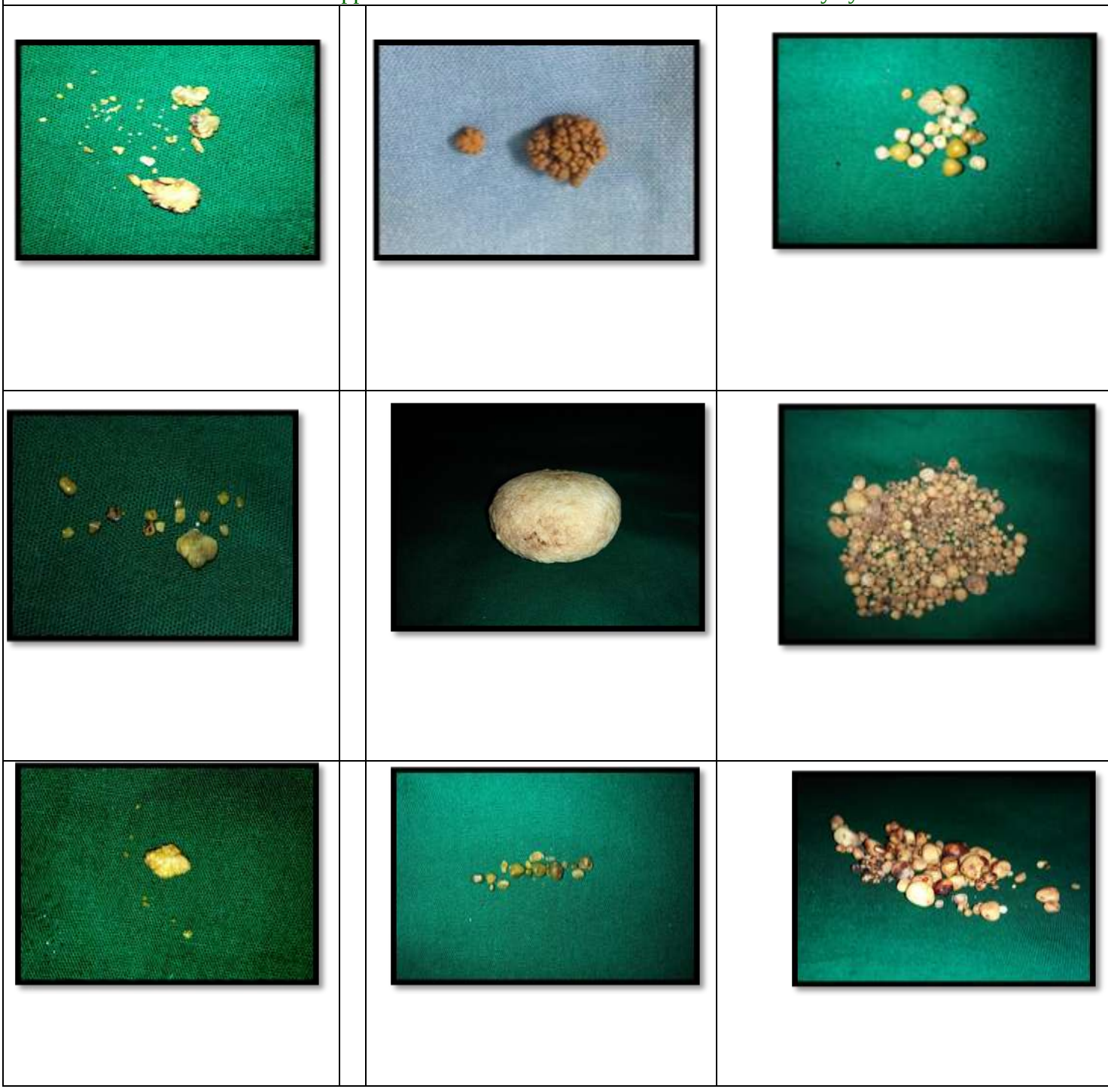
| **Sr No** | Phase/ compound name | Breed | Formula | Nature of stone | Structure | DB ID Number |
|---|---|---|---|---|---|---|
| **10** | $C_2 H_2 Ca O_5$ - (Calcium oxalate monohydrate) | Pug | $C_2 H_2 Ca O_5$ | 1 Phase Crystalline + Amorphus | Monoclinic | 2105964 |
| **11** | $C_2 H_2 Ca O_5$ - (Calcium oxalate monohydrate ) | Spitz | $C_2 H_2 Ca O_5$ | 1 Phase Crystalline + Amorphus | Monoclinic | 2105964 |
| **12** | $Mg (N H_4) (P O_4) (H_2 O)_6$ - (struvite) | Labrador retriever | $H_{13} Mg_1 N_1 O_{10} P_1$ | 1 Phase Crystalline + Amorphus | Orthorhombic | 2106462 |
| **13** | $C_2 H_2 Ca O_5$ - (Calcium oxalate monohydrate ) | Mongrel | $C_2 H_2 Ca O_5$ | 2 Phase Crystalline + Amorphus | Monoclinic | 2105964 |
| | $Ca (C O_2)_2 (H_2 O)_2.2$ - (Calcium carbonate) | | $C_2 H_4.4 Ca_1 O_6 2$ | | Tertagonal | 2310998 |
| **14** | $C_2 H_2 Ca O_5$ - (Calcium oxalate monohydrate) | Pug | $C_2 H_2 Ca O_5$ | 1 Phase Crystalline + Amorphus | Distorted orthorhombic | 2105964 |
| **15** | $C_2 H_2 Ca O_5$ - (Calcium oxalate monohydrate ) | Mongrel | $C_2 H_2 Ca O_5$ | 1 Phase Crystalline + Amorphus | Distorted orthorhombic | 2105964 |
| **16** | $Mg (N H_4) (P O_4) (H_2 O)_6$ - (Struvite) | Pug | $H_{13} Mg_1 N_1 O_{10} P_1$ | 1 Phase Crystalline | Distorted orthorhombic | 2106462 |
| **17** | $C_2 H_2 Ca O_5$ - (Calcium oxalate monohydrate) | Mongrel | $C_2 H_2 Ca O_5$ | 1 Phase Crystalline + Amorphus | Distorted orthorhombic | 2105964 |
| **18** | $Ca (C O_2)_2 (H_2 O)_2.2$ - (calcium carbonate) | Pug | $C_2 H_4.4 Ca_1 O_6 2$ | 3 Phase Crystalline + Amorphus | Tetragonal | 2310998 |
| | Graphite | | C | | Graphanic | 9012233 |
| | $C_{11} H_{28} Cl N O$ -( 7-aminoheptan-2-one) | | C11 H28 Cl N O | | Distorted Tertagonal | 2004934 |
| **19** | $Mg (N H_4) (P O_4) (H_2 O)_6$ - (Struvite) | Labrador | $H_{13} Mg_1 N_1 O_{10} P_1$ | 2 Phase Crystalline | Orthorhombic | 2106462 |
| | Benitoite | | $Ba O_9 Si_3 Ti$ | | Distorted Tetragonal | 9015502 |
| **20** | $Mg (N H_4) (P O_4) (H_2 O)_6$ - (Struvite) | Lhasa apso | $H_{13} Mg_1 N_1 O_{10} P_1$ | 1 Phase Crystalline | Orthorhombic | 2106462 |

**Table.2** Calculi analysis -Quantitative analysis results (XRD method): Lattice information

| Sr No | Breed | Phase/ compound name | a(A) | b (A) | c(A) | alpha (deg) | beta (deg) | gamma (deg) | V(A^3) | Space group |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | Great Dane | $Mg(NH_4)(PO_4)(H_2O)_6$ – (Struvite) | 6.945639 | 6.136024 | 11.20616 | 90 | 90 | 90 | 477.590795 | 31 : Pmn21 |
| **2** | Pug | $Ca(CO_2)_2(H2O)_2.2$ – (Calcium carbonate) | 12.39777 | 12.39777 | 7.367685 | 90 | 90 | 90 | 1132.447556 | 87 : I4/m |
| | | Graphite | 4.703251 | 5.956181 | 4.427659 | 90 | 90 | 90 | 124.03384 | 54 : Pcca |
| | | $C_{11}H_{28}C_lNO$ – (7-aminoheptan-2-one) | 7.735467 | 40.19912 | 4.664484 | 90 | 107.339996 | 90 | 1384.54376 | 14 : P121/a1,unique-b,cell-3 |
| **3** | Labrador Retriever | $Mg(NH_4)(PO_4)(H_2O)_6$ - (struvite) | 6.942781 | 6.137159 | 11.19282 | 90 | 90 | 90 | 476.914297 | 31 : Pmn21 |
| | | Benitoite | 11.24678 | 11.24678 | 9.568958 | 90 | 90 | 120 | 1048.218775 | 159 : P31c |
| **4** | Lasa Apso | $C_2H_2CaO_5$ -(Calcium carbonate) | 9.987673 | 7.302072 | 6.2981 | 90 | 107.07 | 90 | 439.090153 | 12 : I12/m1,unique-b, cell-3 |
| **5** | Pug | $Mg(NH_4)(PO_4)(H_2O)_6$ - (Struvite) | 6.957194 | 6.141543 | 11.22027 | 90 | 90 | 90 | 479.418801 | 31 : Pmn21 |
| | | 1,15-pentadecanediol | 7.310404 | 42.61065 | 5.063505 | 90 | 90 | 90 | 1577.286989 | 19 : P212121 |
| **6** | Labrador Retriever | $Mg(NH_4)(PO_4)(H_2O)_6$ - (Struvite) | 6.950281 | 6.144647 | 11.21288 | 90 | 90 | 90 | 478.868483 | 31 : Pmn21 |
| **7** | Mongrel | $Ca(CO_2)_2(H_2O)_2.2$ – (Calcium carbonate) | 12.37651 | 12.37651 | 7.355816 | 90 | 90 | 90 | 1126.749295 | 87 : I4/m |
| **8** | Dalmatian | $MRHBrH_20$ – (Gabazine bromide) | 7.870705 | 11.8689 | 16.62125 | 90 | 92.568001 | 90 | 1551.142194 | 14 : P121/c1,unique-b,cell-1 |
| **9** | Pug | $C_2H_2CaO_5$ -(Calcium oxalate monohydrate) | 10.00382 | 7.313878 | 6.308282 | 90 | 107.07 | 90 | 441.223327 | 12 : I12/m1,unique-b,cell-3 |
| | | $Ca(CO_2)_2(H_2O)_2.2$ - (Calcium Carbonate) | 12.41292 | 12.41292 | 7.361348 | 90 | 90 | 90 | 1134.240822 | 87 : I4/m |
| **10** | Pug | $C_2H_2CaO_5$ - (Calcium oxalate monohydrate) | 9.985276 | 7.30032 | 6.296588 | 90 | 107.07 | 90 | 438.774114 | 12 : I12/m1,unique- |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | b,cell-3 |
| **11** | Spitz | $C_2 H_2 Ca O_5$ - (Calcium oxalate monohydrate ) | 9.98327 | 7.298853 | 6.295323 | 90 | 107.07 | 90 | 438.509703 | 12 : I12/m1,unique-b,cell-3 |
| **12** | Labrador retriever | Mg ($N H_4$) ($P O_4$) ($H_2 O)_6$ - (struvite) | 6.953131 | 6.133929 | 11.20025 | 90 | 90 | 90 | 477.690591 | 31 : Pmn21 |
| **13** | Mongrel | $C_2 H_2 Ca O_5$ - (Calcium oxalate monohydrate ) | 9.997383 | 7.309172 | 6.304223 | 90 | 107.07 | 90 | 440.372108 | 12 : I12/m1,unique-b,cell-3 |
| | | Ca ($C O_2)_2$ ($H_2 O)_2$.2 - (Calcium carbonate) | 12.38554 | 12.38554 | 7.362706 | 90 | 90 | 90 | 1129.451074 | 87 : I4/m |
| **14** | Pug | $C_2 H_2 Ca O_5$ - ( Calcium oxalate monohydrate) | 9.990467 | 7.304115 | 6.299861 | 90 | 107.07 | 90 | 439.458747 | 12 : I12/m1,unique-b,cell-3 |
| **15** | Mongrel | $C_2 H_2 Ca O_5$ - (Calcium oxalate monohydrate ) | C2 H2 Ca O5 | 9.982843 | 7.298541 | 6.295 054 | 90 | 107.07 | 90 | 12 : I12/m1,unique-b,cell-3 |
| **16** | Pug | Mg ($N H_4$) ($P O_4$) ($H_2 O)_6$ - (Struvite) | 6.945639 | 6.136024 | 11.20616 | 90 | 90 | 90 | 477.590795 | 31 : Pmn21 |
| **17** | Mongrel | $C_2 H_2 Ca O_5$ - (Calcium oxalate monohydrate ) | 9.987673 | 7.302072 | 6.2981 | 90 | 107.07 | 90 | 439.090153 | 12 : I12/m1,unique-b,cell-3 |
| **18** | Pug | Ca ($C O_2)_2$ ($H_2 O)_2$.2 - (calcium carbonate) | 12.39777 | 12.39777 | 7.367685 | 90 | 90 | 90 | 1132.447556 | 87 : I4/m |
| | | Graphite | 4.703251 | 5.956181 | 4.427659 | 90 | 90 | 90 | 124.03384 | 54 : Pcca |
| | | $C_{11} H_{28}$ Cl N O -( 7-aminoheptan-2-one) | 7.735467 | 40.19912 | 4.664484 | 90 | 107.339996 | 90 | 1384.54376 | 14 : P121/a1,unique-b,cell-3 |
| **19** | Labrador | Mg ($N H_4$) ($P O_4$) ($H_2 O)_6$ - (Struvite) | 6.942781 | 6.137159 | 11.19282 | 90 | 90 | 90 | 476.914297 | 31 : Pmn21 |
| | | Benitoite | 11.24678 | 11.24678 | 9.568958 | 90 | 90 | 120 | 1048.218775 | 159 : P31c |
| **20** | Lhasa apso | Mg ($N H_4$) ($P O_4$) ($H_2 O)_6$ - (Struvite) | 6.950281 | 6.144647 | 11.21288 | 90 | 90 | 90 | 478.868483 | 31 : Pmn21 |

**Plate.1** Appearance of various calculi retrieved from urinary system



In conclusion x-ray diffractometry revealed struvite calculi ( 40% ) to be the most common followed by calcium carbonate (25%), calcium oxalate monohydrate (10%), 7-aminoheptan-2-one (10%), benetoite (10%), graphite (10%), gabazine bromide (5%) and 1,5-pentadecanediol (5%. Out of 20 samples 13 calculi revealed single phase, 5 samples double phase and 2 samples had 3 phase compounds in the calculi.

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