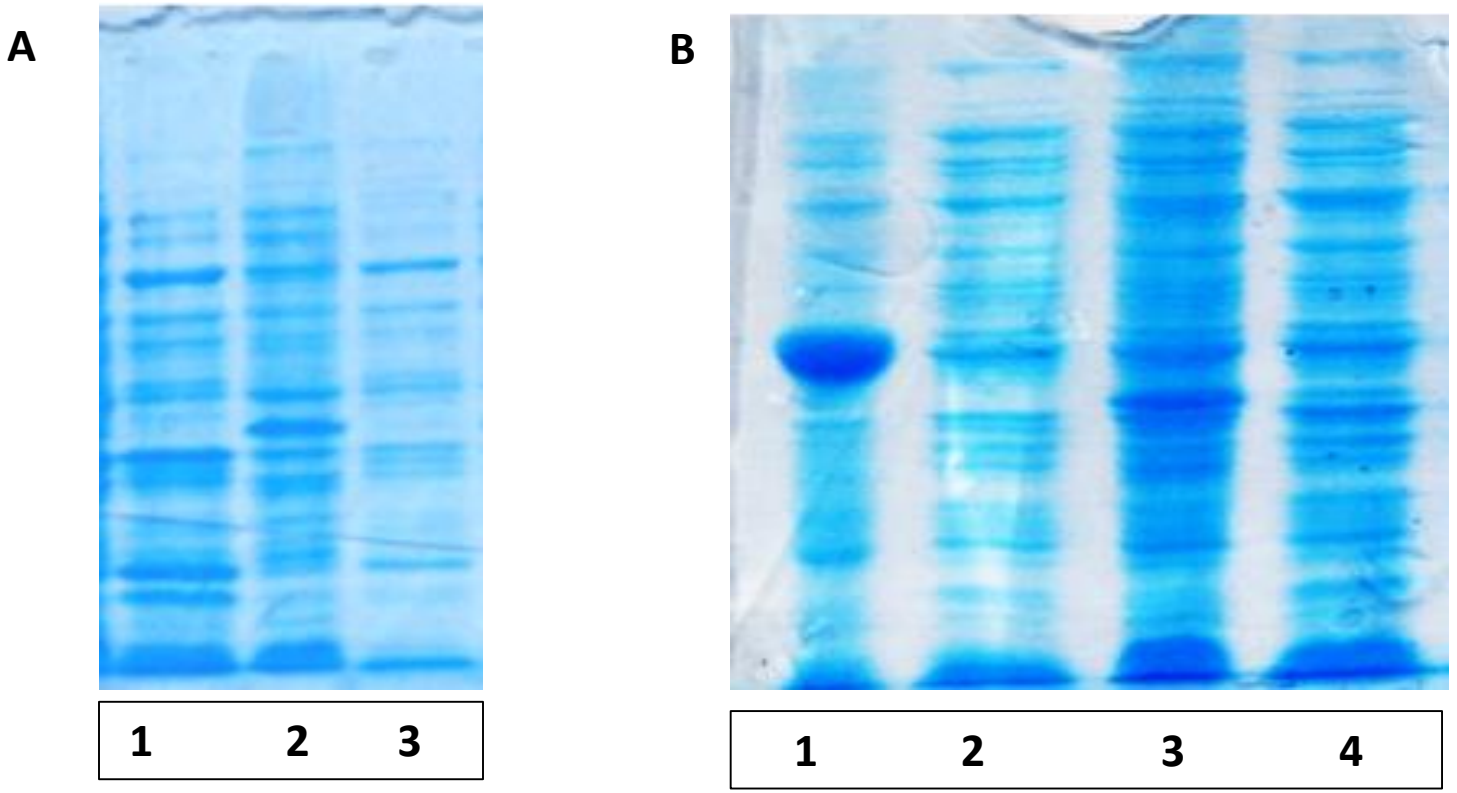


Fig 2. A. Impact of different temperatures on MEVE7 expression level in *BL21-gold*. Lane 1: BL21-gold/pET28- MEVE7 without IPTG induction; lane 2: at 37°C; Lane 3: at 25°C; M: Molecular weight marker. The arrow indicates the expressed MEVE7. SDS-PAGE analysis demonstrated that protein expression was decreased at 25°C compared to 37°C, and no protein expression was observed at 20°C (data not shown).**B**. Impact of different concentrations of IPTG on MEVE7 expression level at 37°C. Lane 1: Positive control protein (45 kD); Lane 2: BL21/pET28- MEVE7 without IPTG induction; Lane 3: 0.1 mM IPTG; Lane 4: 1.0 mM IPTG; M: Molecular weight marker. The arrow indicates the expressed MEVE7. 1.0 mM concentration of IPTG decreased protein expression (at 37°C).

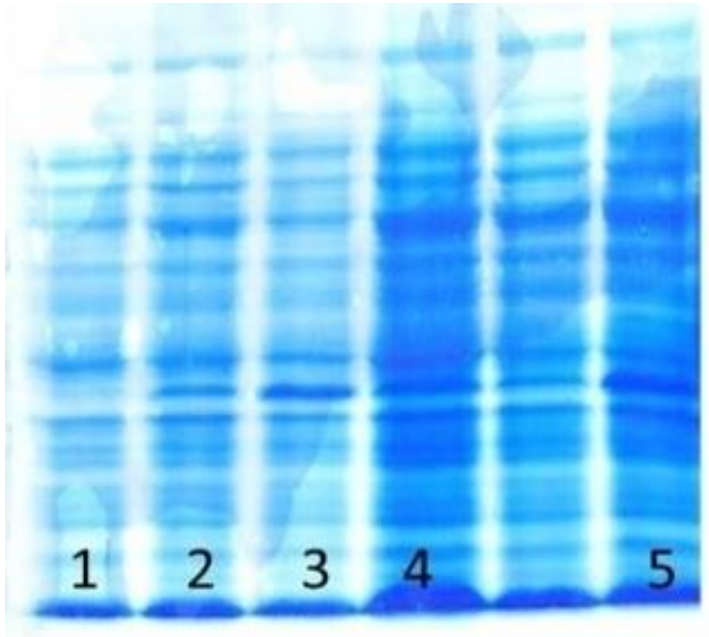


Fig 3. Impact of various incubation times after induction on MEVE7 expression level in *BL21-gold*. Lane1: 1 hr after incubation; Lane 2: 2 hrs after incubation; Lane 3: 4 hrs after incubation; Lane 4: 8 hrs after incubation, Lane 5: 16 hrs after incubation; M: Molecular weight marker. The arrow indicates the expressed MEVE7. The best induction time is 4 hrs, and further reduced of MEVE7 yield.

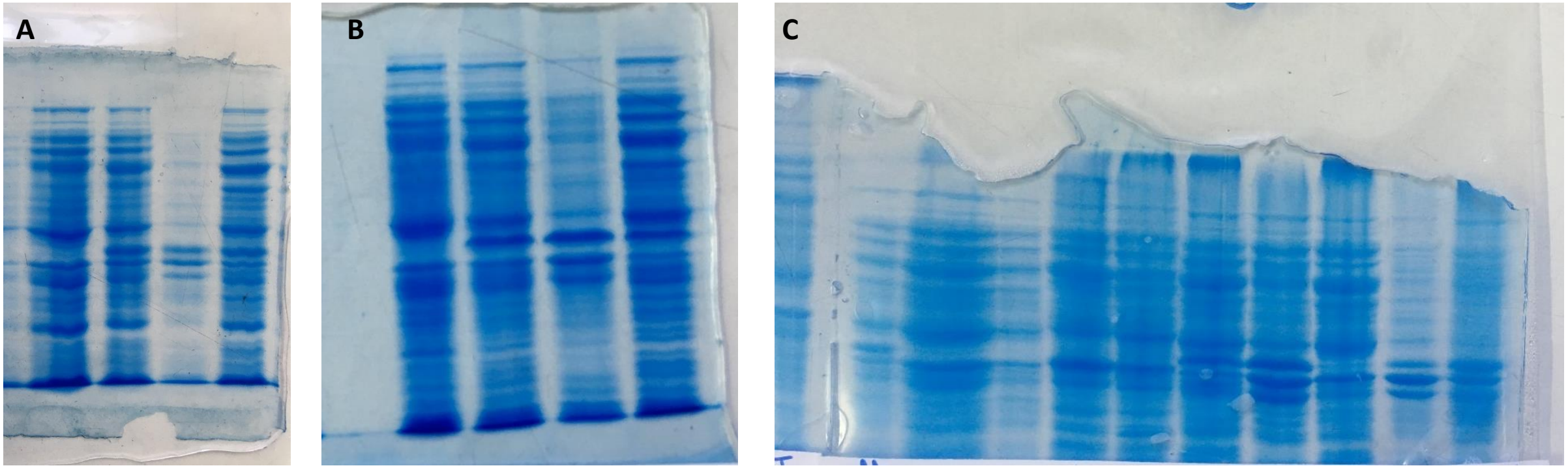
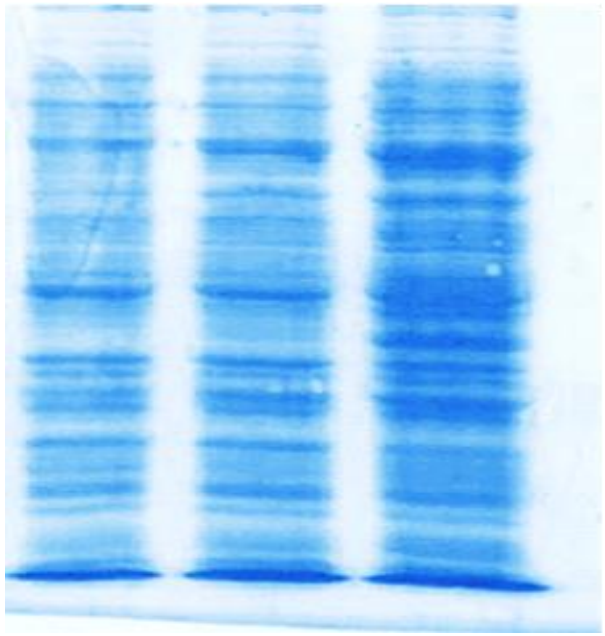
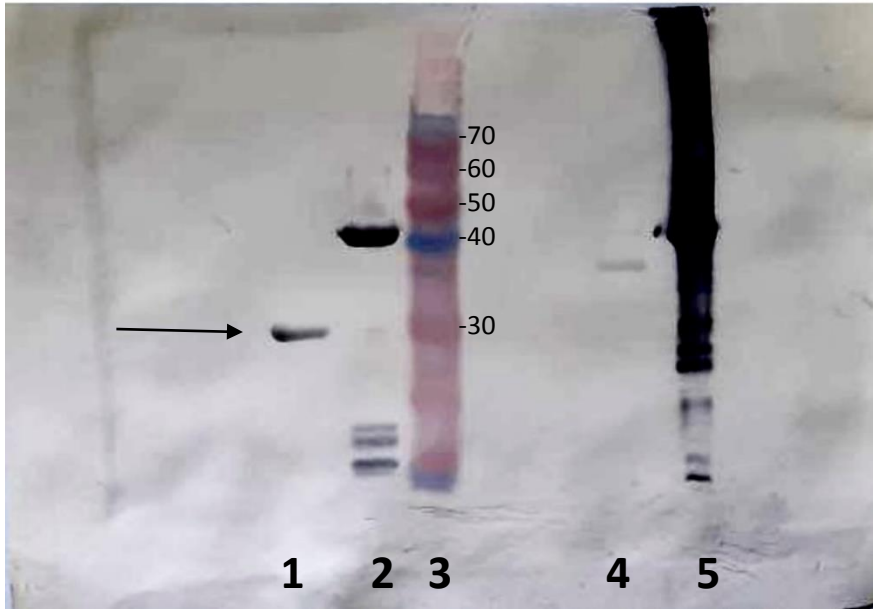


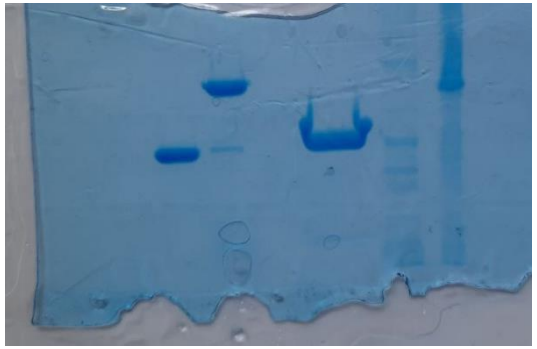
Fig 4. Expression of MEVE7 in different strains of *E. coli* in the presence of 0.1 mM IPTG (OD600=0.5) after 4h induction at 37 ℃. **A**: Expression of MEVE7 in *Rosetta*, **B**: Expression of MEVE7 in *BL21-gold*, **C**: Expression of MEVE7 in *BL21 (DE3)*. B.I: Before induction, A.I: After induction, I: insoluble fraction, S: soluble fraction.

A



B





The gel that was used for western blot.

Fig 5. A. SDS-PAGE analysis of pET28-MEVE7 expression in *E. coli BL21-gold*. Lane 1: BL21/pET28-MEVE7 without IPTG induction; lanes 2 & 3: induced with IPTG for 2, 16 h respectively. The arrow indicates the expressed MEVE7. **B**. Western blot analysis (using anti-His tag antibody) of MEVE7. Lane 1: MEVE7; M: Molecular weight marker (Thermo Fisher).

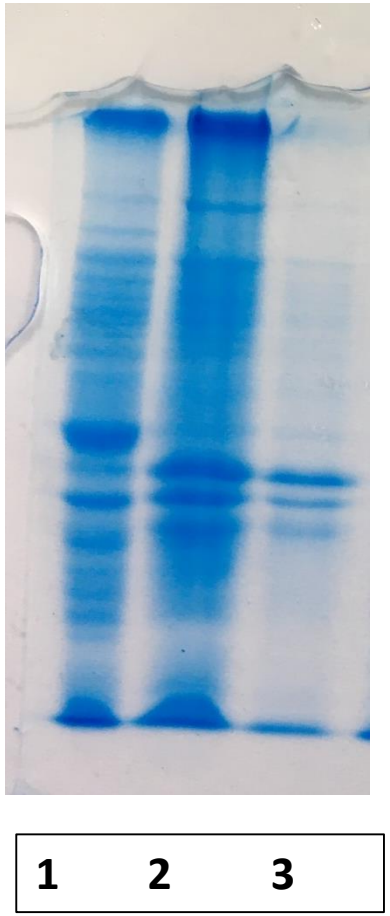


Fig 6. Isolation of the inclusion bodies with Tris buffer (pH: 8.5). Lane 1: Positive control (45 kDa); Lane 2: MEVE7; Lane 3: washed IB. The arrow indicates the expressed MEVE7.

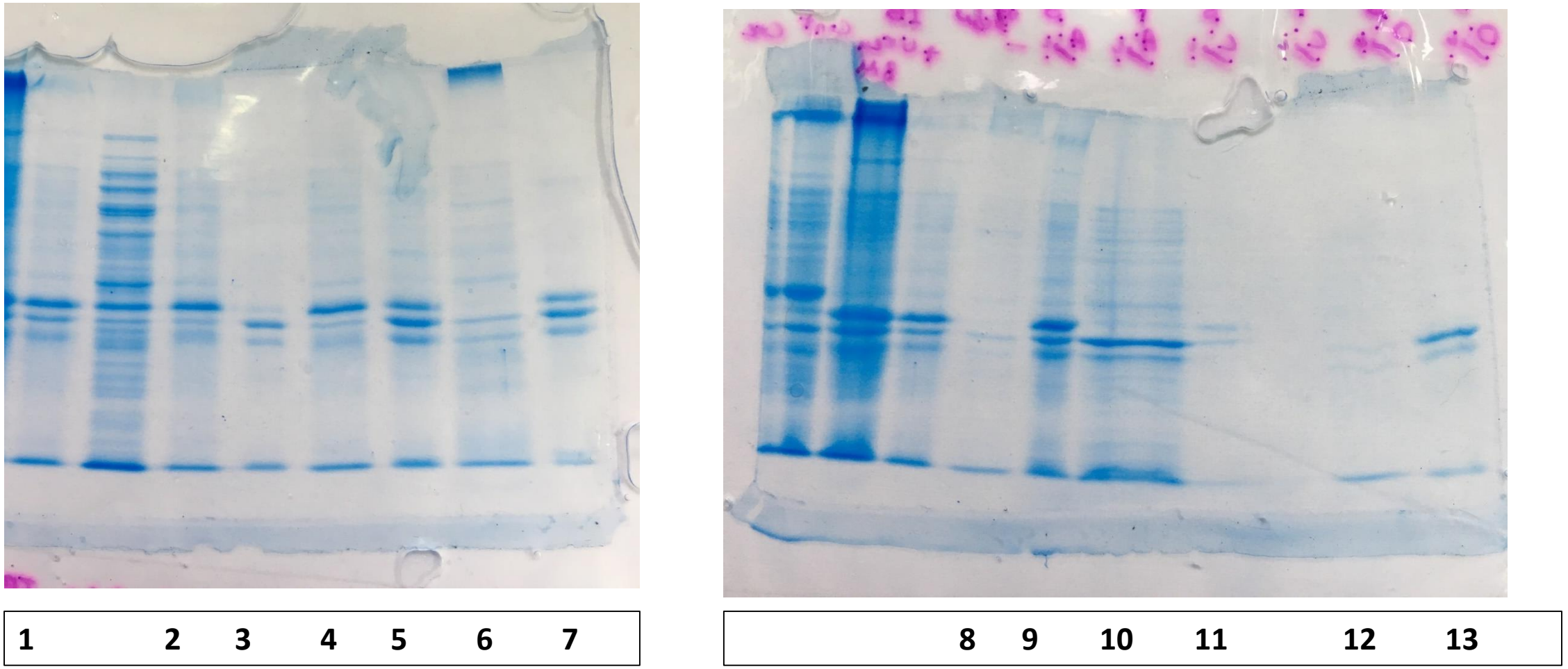


Fig7. SDS-PAGE of different solvents A–F effect on the solubility of MEVE7 inclusion bodies. Buffers are described in Table1.; Lane 1: washed IB; Lane 2: Soluble fraction (S) after solubilization with buffer A; Lane 3: precipitate fraction (P) remained after solubilization with buffer A; Lane 4: S of buffer C; Lane 5: P of buffer C; Lane 6: S of buffer D; Lane 7: P of buffer D; Lane 8: S of buffer E; Lane 9: P of buffer E; Lane 10: S of buffer F, Lane 11: P of buffer F; Lane 12: S of buffer B; Lane 13: P of buffer B; M: Molecular weight marker. (S= supernatant, P= precipitate). The highest levels of soluble form were obtained in buffer C and then buffer A. Other buffers used for solubilization were not so effective.