SUMMARY AND CONCLUSIONS
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*Cucumis sativus* belongs to the cucurbit family Cucurbitaceae and commonly known as a Cucumber. In Ayurveda, the fruits, seeds and leaves of cucumber plants are extensively used as medicine for various skin disorders such as inflammation under the eyes, suntan and are supposed to endorse healing, cooling, emollient, soothing and anti-itching effect of aggravated skin, fever, insomnia, bronchitis, jaundice, hemorrhages and extended cosmetic effects. All parts of cucumber fruit are being used for various purposes, but the fruit, sap although always associated with the fruit pulp, its biochemical and medicinal properties are unexplored. Cucumber extract/slices are extensively used to arrest hemorrhage and to promote healing effects in wounds. Therefore, it was hypothesized that, the proteases present in the fruit, sap/peal might play role in stopping the bleeding of fresh wounds by promoting clot formation.

The cucumber saps extract (CSE) showed proteolytic activity on various protein substrates such as casein, gelatin, hemoglobin, and BSA with the specific activities of $2.27 \pm 0.6$, $1.4 \pm 0.6$, $1.13 \pm 0.3$ and $0.27 \pm 0.0$ units/mg/min respectively. The activity seen was in the widest pH range from pH 5.5 to 12.5 and with two pH optima respectively at pH 8.5 and 10.5. The temperature versus activity profile revealed the optimum temperature of 45°C. The proteolytic activity was inhibited by various inhibitors, the percent inhibition varied as EDTA (29 ± 2.5%), EGTA (27 ± 5%), 1, 10, phenanthroline (35 ± 5%), PMSF (40 ± 7%) and IAA (12 ± 5%). Further the sensitivity towards metal ions, solvents and detergents were studied and they revealed varied extent of sensitivity in which Mg$^{2+}$, Cu$^{2+}$, and Li$^+$ enhanced the activity of CSE, while K$^+$, Zn$^{2+}$ and Fe$^{2+}$ decreased the activity significantly whereas Na$^+$, Ca$^{2+}$ and Co$^{2+}$ did not affect, however Hg$^{2+}$ abolished the proteolytic activity of
CSE. The stability of CSE proteolytic activity towards organic solvents varied significantly, at 0.5% in the reaction mixture. Solvents such as DMSO and isopropanol enhanced the activity by 24 ± 7% and 12 ± 5%, while hexane and benzene decreased the activity by 6 ± 2.5% and 18 ± 2.5% respectively as compared to the control value whereas ethanol had no effect on the activity. Similarly, at 0.5%, SDS and Triton X100 decreased activity by about 30 ± 8% and 20 ± 4% respectively was observed.

CSE was extensively characterized for its effect on hemostasis and wound healing. It reduces the re-calcification time, prothrombin time and clotting time of the factor VII deficient plasma, suggesting that it is procoagulant in nature and shows factor VIIa like activity and interfered in the tissue factor (extrinsic) pathway of blood coagulation process. The metalloprotease activity of CSE is responsible for the procoagulant activity which was confirmed by using known protease inhibitors such as EGTA, EDTA and 1,10, phenanthroline. CSE proteolytic activity cleaved all the chains of human fibrinogen; however, the Bβ chain is hydrolyzed over Aα chain while the γ chain is hydrolyzed with a less preference.

Generally the fibrin degradation was due to the plasmin, where plasminogen is converted to plasmin by plasminogen activators. Urokinase is one of the known activator of plasminogen to form plasmin, which will degrade fibrin clot. In our study the urokinase didn’t degrade azocasein itself/alone when incubated independently with plasma contaminated with fibrinogen, while CSE hydrolyzed azocasein suggesting the plasminogen activation property and this was abolished by IAA and thus the role of Cysteine protease in the process. This is the first study reporting plasminogen activation by the cysteine protease. CSE hydrolyzed all chains of fibrin,
Summary and Conclusions

where α polymer and α chain are hydrolyzed in preference nearly to a similar extent over β chain and γγ dimmers, and this was abolished by PMSF and IAA and thus the role of serine and cysteine proteases in fibrin hydrolysis.

Interestingly the CSE inhibited platelet aggregation of human PRP completely which was induced by epinephrine, collagen and ADP in the order epinephrine > collagen > ADP with the pertinent IC$_{50}$ of 11 ± 5, 20 ± 10 and 22 ± 12 µg/ml and this was abolished by PMSF suggesting that the serine protease was responsible for inhibition of platelet aggregation/ anticoagulant activity of CSE. For the first time using CSE we reporting the factor VII-like activity of a CSE protease(s) with the two contrasting effects on hemostasis process. The properties such as factor VIIa-like activity and activator of prothrombin complex, counted for pro-coagulation while, fibrinolytic, activation of plasminogen and inhibition of ADP, collagen and epinephrine induced platelet aggregation of PRP leads to anti-coagulation activity.

Hemostasis and wound healing are interrelated; during wound healing arrest of hemorrhage and removal of scar is important, CSE enhanced the formation of clots and dissolution of the clot. The wound healing activity was performed using the albino mouse model. The wound contraction /closure percentage was increased in case of wound treated with CSE compared to negative control, positive control and protease control. Histology of the excision wound also showed normal epithelization, adnexa, and fibrosis within the dermis. It increases the hexosamine, hexuronic acid and hydroxyproline levels of granulation tissue which indicates the amplified rate of wound healing. In addition, the histology of granulation tissue sections of CSE and Neosporin treated mice showed a profusion of collagen tissue and neovascularisation with a small amount of inflammatory cells on the 14th day of treatment compared to
Summary and Conclusions

NC and B-CSE which suggests the augmented wound healing process. The initial increased SOD, CAT, GSH levels and their decreased pattern after 14\textsuperscript{th} day and the decreased levels of NO, LPO and MPO at 21\textsuperscript{st} day in CSE treated granulation tissue suggests the significant antioxidant activity. Reduction of free radicals and MPO levels could avoid oxidative damage and endorse the healing processes. CSE is devoid/free from toxicity such as hemorrhage, edema and myonecrosis.

In conclusion, the data from this study clearly suggest that the proteases of CSE strongly interfere in the hemostasis process through its procoagulant, factor VIIa-like, and fibrinogenolytic activities. The clot dissolution and inhibition properties of CSE find immense value to explore these proteases as therapeutic tools or lead molecules in the treatment and management of life threatening conditions in clinical field such as stroke and angina (cardiac pain) and to treat severe chronic wounds without any adverse effects on the patient.