Summary & Conclusion

Chapter 6

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In this study, we investigated in-vitro the anticoagulant properties of methanolic extracts from *Citrus medica* fruit, *Tecomella undulata* bark, and *Sesamum indicum* seeds using PT and APTT assay. Both *Citrus medica* fruit and *Tecomella undulata* bark extracts showed prolonged clotting time, PT, and APTT, prompting further investigation. These extracts underwent solvent-solvent partitioning, yielding fractions that were tested for *in vitro* antioxidant and anticoagulant activity. Among these fractions, the ethyl acetate fraction of *Citrus medica* fruit and the butanol fraction of *Tecomella undulata* bark exhibited the most significant prolongation of clotting time, PT, and APTT, thus warranting in vivo anticoagulant activity assessment. Additionally, qualitative and quantitative phytochemical screenings revealed the presence of therapeutic phytochemicals, confirmed by GC-MS analysis. Moreover, these fractions demonstrated considerable *in vitro* antioxidant activity, suggesting their potential in managing primary hemostasis disorders.

In the *in vivo* anticoagulant activity assessment using a rat model, the ethyl acetate fraction of *Citrus medica* fruit significantly prolonged clotting time, PT, and APTT at 200 mg/kg dose over seven days, while the butanol fraction of *Tecomella undulata* bark achieved similar effects at 200 mg/kg but for three days only. These findings indicate the potential anticoagulant activity of *Citrus medica* fruit extracts, particularly the ethyl acetate fraction. Furthermore, LCMS/MS analysis identified flavonoids like rutin, quercetin, and gallic acid in the ethyl acetate fraction, contributing to its antioxidant and anticoagulant properties.

Isolation and characterization of bioactive compounds from the ethyl acetate fraction of *Citrus medica* revealed the presence of ferulic acid, confirmed by HPTLC and FTIR analyses. The therapeutic effects observed in *Citrus medica* fruit extracts and their ethyl acetate fractions may be attributed to the presence of flavonoids, including ferulic acid, which potentially inhibit protease enzymes involved in the coagulation cascade and activate natural anticoagulant pathways. These effect resemble those of warfarin, indicating comparable anticoagulant effects within a narrow concentration range of activity.

In summary, *C.medica* is a traditional medicinal plant. The various components and their conventional pharmacological activity were the primary subjects of the earlier research on *C. medica*. The gallic acid (514.543 ng/mg), quercetin (67.839 ng/mg), rutin (53.691 ng/mg), and ferulic acid are present in the flavonoid-rich bioactive ethyl acetate fraction of

Citrus medica which were plentiful in the plant from which they were isolated and separated. These flavonoids are responsible for *Citrus medica's* ethyl acetate fraction's anticoagulant properties both *in-vitro* and *in-vivo*. Their potential as a substitute therapy for hemostasis problems is suggested by the fact that their effect is similar to that of warfarin.*C.medica* is a new, promising medication that can be used to treat a variety of coagulation issues. People who are at high risk of hemostasis or other bleeding disorders may benefit from using it. The techniques for isolation and separation were straightforward to use. They may have promising futures in pharmaceuticals. They can satisfy the demands of the current antithrombotic medication market because they are affordable, secure, and efficient.