ABSTRACT

Introduction:

Blood coagulation is a vital, strictly controlled mechanism that creates clots quickly. On the other hand, blood coagulation abnormalities are frequently noted in several medical disorders. To find new uses for this plant, this study investigated the anticoagulant activity of methanolic extracts of *Citrus medica* fruit, *Tecomella undulata* bark, and *Sesame indicum* seed using in vitro techniques.

Material and method:

70% methanol was used to extract the dried and powdered *Citrus medica* fruit, *Tecomella undulata* bark, and *Sesame indicum* seed. The concentrated dried crude extracts (CMME, TUME, and SIME) were then subjected to an analysis of their in-vitro anticoagulant activity on blood coagulation profile, including activated partial thromboplastin time (aPTT), prothrombin time (PT), and clotting time (CT). Petroleum ether, benzene, ethyl acetate, and butanol were used in a liquid partitioning process for the CMME and TUME extracts. The fractions were examined in vitro for their antioxidant potential, blood coagulation profile, clotting time (CT), prothrombin time (PT), and activated partial thromboplastin time (aPTT) at varying concentrations (2.5–10 mg/mL). The greatest anticoagulant fraction (the butanol fraction of TUME (TUBUF) and the ethyl acetate fraction of CMME (CMEAF)) was subjected to GCMS analysis. Using an animal model for seven days, the in-vivo anticoagulant activity of CMEAF (100, 200, and 400 mg/kg) was determined. The bioactive chemical constituents or constituents responsible for the anticoagulant activity were discovered by FTIR and HPTLC after being isolated by preparative TLC. Using the LCMS/MS technique, the bioactive molecule was quantified.

Result:

Prothrombin time (PT) (20.1, 18.83 seconds) activated partial thromboplastin times (APTT) (93.67, 63.67 seconds), and clotting time (18.29 ± 0.8, 15.37 ± 0.91 minutes) were all significantly ($p<0.05$) extended when administered with CMME and TUME 200 mg/kg. At a concentration of 7.5 mg/mL, the ethyl acetate fraction of *Citrus medica* (clotting time: 21.85 minutes; PT: 91.33 seconds; APTT: 114.67 seconds) and the butanol fraction of *Tecomella undulata* (17.95 minutes; PT: 48.33 seconds; APTT: 114.67 seconds) showed

the highest prolongation significantly (P<0.001) prolonged effect. Ten compounds in *Tecomella undulata's* butanol fraction and fifteen compounds in *Citrus medica*'s ethyl acetate fraction have antioxidant and anticoagulant activity, according to GCMS analytical data. Additionally, when compared to warfarin as standard, CMEAF prolongs clotting time, PT, and APTT in the in-vivo model at a dose of 200 mg/kg for five days. It was found that the amounts of gallic acid, quercetin, and rutin in CMEAF were 514.543 ng, 67.839 ng, and 53.691 ng, in that order. Ferulic acid, the isolated chemical, was verified using an FTIR chromatogram and an HPTLC fingerprint.

Conclusion:

The results of the study demonstrate the potential of *Tecomella undulata*, particularly its butanol fraction, as an antioxidant and anticoagulant, as well as a promising and unexplored source of bioactive compounds (pentanedioic acid, 2-oxo-dimethyl ester) with potential therapeutic applications. The ethyl acetate fraction of *citrus medica*, which is rich in flavonoids, may include bioactive substances such as gallic acid, quercetin, rutin, and ferulic acid that have anticoagulant qualities. This offers a possible route for the creation of brand-new anticoagulant medications. It can be investigated further to create novel treatments that address a range of medical issues.

Keywords: Blood coagulation, clotting time, prothrombin time, solvent partitioning, *Citrus medica* fruit, *Tecomella undulata* bark, and *Sesamum indicum* seed, GCMS, HPTLC, FTIR.

Graphical Abstract