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RESEARCH ARTICLE

IN-VITRO ANTICOAGULANT EFFECT OF AERIAL PARTS EXTRACTS OF YEMENI *FAGONIA SCHWEINFURTHII* HADIDI

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Abstract

Background: Blood coagulation is a quick and effective process that results in the creation of clots, which demands to monitor. Many illness disorders include an abnormality in blood coagulation. This study examined the *in vitro* effects of methanol, ethyl acetate, and n-hexane extracts from aerial parts of *Fagonia schweinfurthii* Hadidi on healthy human volunteers' blood coagulation.
Methods: The Secondary metabolites were extracted from dried and crushed *F. schweinfurthii* aerial parts using n-hexane, ethyl acetate, and methanol, respectively. Additionally, the extracts were tested *in vitro* at different concentrations (10-100 µg/ml) on the blood coagulation profile, prothrombin time (PT), and activated partial thromboplastin time (a PTT) of apparently healthy human volunteers.
Results: Methanol, ethyl acetate, and n-hexane extracts of *F. schweinfurthii* aerial parts significantly (p>0.05) prolonged PT and PTT in the blood of healthy human volunteers with Ethyl acetate and methanol extracts recorded the largest prolongation of PT and PTT correspondingly. The highest PT and PTT prolongation was achieved at 100 µg/ml, and the least prolongation time was obtained at 10 µg/ml.
Conclusion: These findings displayed that *F. schweinfurthii* aerial parts contain phytochemical constituents with anticoagulant characteristics and could be used to treat blood clotting disorders.
Keywords: Blood coagulation, *F. schweinfurthii*, Partial thromboplastin time, Prothrombin time.

INTRODUCTION

Medicinal plants are thought to be a rich source of ingredients for medication development and production. Furthermore, these plants play a significant role in the formation of human cultures all throughout the world. Additionally, some plants are thought to be important sources of nutrition, and as a result, some plants are indicated for their therapeutic potential[1]. Many secondary metabolites produced by plants have important biological activity. Anticoagulant action may be highlighted among these. Anticoagulants treat and prevent blood clots by interacting with the body's natural blood-clotting system. Anticoagulants treat patients with pulmonary embolism (PE), deep vein thrombosis (DVT), blood clots in the arteries or veins, atrial fibrillation, which causes strokes, and mechanical heart valves[2].

F. schweinfurthii belongs to the family Zygophyllaceae[3]. It is annual to biennial, up to 25 cm tall, spiny, erect, undershrub, with more and less granular leaves; thin, terete, triangular, glabrous branches; opposite, 1-3 foliate leaves. Petioles range from 3 to 30 mm in length, are deeply striated, and are very slender; stipules have two pairs of sharp, very short petioles. Geographically it is distributed in India, Pakistan, Iran, Eritrea, Ethiopia, Sudan, Somalia, and Kenya[4,5]. In Yemen, *F. schweinfurthii* is distributed in Tehama, Shara'b, Muthaikhira, Aden, Lahj, Abyan, Qatabah, Demt, al Hus'ein, Ashu'ib, Juban, Amran, Raydah, Huth, Haddah, E of Rada, Hadramout, Socotra[6,7].
People living in desert regions have traditionally utilized the plant to treat various conditions, including ear infections, skin eruptions, heal sores, skin illnesses, fever, and pain relief[8]. They have traditionally been used to treat hemorrhoids, inflammation, ulcers, leprosy, open wounds, and fever in internal and

external formulations. When powder from the entire parts of *F. schweinfurthii* is applied to boils and skin eruptions, it induces healing; when the whole plant is boiled in water, its bath is beneficial for allergies and other skin problems; and the decoction is administered orally as a blood purifier[9]. *Fagonia* sp. has been reported to have anticancer, antibacterial, antiviral, analgesic, anti-inflammatory, antipyretic, coolant, antioxidant, and thrombolytic activities[10]. Phytochemical screening revealed that *F. schweinfurthii* extracts contain alkaloids, cardiac glycolsides, flavonoids, carbohydrates, tannins, saponins, steroids, and amino acids[5]. The anticoagulant activity of *Fagonia arabica* extracts (water, ethanol, methanol, isopropyl alcohol (IPA), butanol, ethyl acetate, acetone, chloroform, petroleum ether, and hexane) was studied *in vitro* and revealed that the aqueous extract and its fifth fraction had the greatest anticoagulant effect (31 minutes and 27 minutes, respectively)[15]. Furthermore, Ismail *et al.*,[14] demonstrated that *Fagonia cretica* had marked anticoagulant activity with a coagulation time of 86.9 seconds. The effects of *F. schweinfurthii* on blood coagulation have received little to no scientific attention. As a result, this study aims to investigate the possibility and potential of *F. schweinfurthii* as a medicinal plant for the treatment of blood coagulation disorders.

MATERIALS AND METHODS

Drugs and chemicals

The reagents used were all of the analytical quality. Prothrombin Time (PT) and activated partial thromboplastin time (PTT) reagent kits were obtained from SEIMENS.

Plant Material:

F. schweinfurthii aerial parts were collected in September 2019 from local areas in Sana'a, Yemen. Dr. Hassan Ibrahim identified the plant in the Biology Department, Sciences College, Sana'a University.

Preparation of plant extract:

One kilogram of fresh aerial parts was properly rinsed with tap water, dried at room temperature, and then using an electric blender, reduced to a fine powder. The powder was stored at cool place and protected from light prior to future uses. In 3.76L conical flasks, powdered aerial parts weighing 752g were sequentially soaked in hexane, ethyl acetate, and methanol. The samples were immersed at room temperature for three to seven days with continuous agitation. These procedures were repeated three times to obtain higher extraction yields. The extracts were then filtered, concentrated in a rotary evaporator at 40°C, and dried in an oven at 37°C. Then the dried extracts were weighed and kept at 4°C for later investigation.

***In vitro* anticoagulant activity assay:**

Using the traditional coagulant tests PT and PTT, the anticoagulant activity of *F. schweinfurthii* aerial parts extracts was examined. Nine parts of healthy human blood were drawn into a one-part sample which would be measured. Heparin and extracts of *F. schweinfurthii* aerial parts were made in different concentrations (100, 75, 50, 25, 10, and 10g/ml), and all of the samples were dissolved in a 0.9% (w/w) solution of NaCl. Plasma alone was the negative control, whereas heparin was a positive control. For the PT assay, citrated normal plasma was combined with a sample solution, which was then incubated for 3 minutes at 37°C. After pre-incubating the PT test reagent for 3 minutes at 37°C, 0.20 ml was added to the mixture, and the clotting time was noted. Then, PTT assay reagent 0.10 ml, pre-incubated for 3 min at 37°C, was added to the mixture and incubated for 5 min at 37°C. After that, 0.10 ml $CaCl_2$ (0.025 mol/L) pre-incubated for 3 min at 37ºC was added, and clotting time was recorded[12]. The experiment was carried out in triplicate.

Statistical analysis

The data was analyzed using SPSS version 26. The mean ±SD from three distinct trials was used to express all the results performed in triplicate. One Way-ANOVA test was used to detect statistical differences between the means in all studies, Followed by Dunnett's multiple comparison test. The statistically significant difference was denoted by $p<0.05$, $p<0.01$, and $p<0.001$.

RESULTS

The anticoagulant activity of methanol, n-hexane, and ethyl acetate extracts of *F. schweinfurthii* aerial parts was determined *in vitro* using the traditional coagulation assays prothrombin time (PT) and activated partial thromboplastin time (a PTT) assays, with the use of normal citrated human plasma as a negative control and heparin as a reference. Many different concentrations (10, 25, 50, 75, and 100 µg/ml) of *F. schweinfurthii* aerial parts methanol, ethyl acetate, and n-hexane extract were prepared, and the results are given in Table 1 and Table 2 and Figure 1 and Figure 2).

Table 1: Results of *in vitro* anticoagulant activity assay (PT) in a sec of *F. schweinfurthii* aerial parts extracts.

Sample concentration (µg/ml)	Methanol extract	Ethyl acetate extract	Hexane extract	Heparine
10	12.91±0.22[c]	13.11±0.28[b]	12.74±0.74	63.32±1.70[a]
25	14.11±0.48[a]	16.07±0.17[a]	15.22±0.92[b]	93.52±0.60[a]
50	19.21±0.51[a]	21.88±0.23[a]	21.03±0.75[a]	115.02±1.14[a]
75	23.79±0.33[a]	25.69±0.46[a]	24.93±1.01[a]	139.73±2.87[a]
100	26.59±0.57[a]	29.44±0.77[a]	28.08±1.85[a]	157.06±1.98[a]
Control	11.80±0.07	11.80±0.07	11.80±0.07	11.80±0.07

Note: [a]*p* value <0.001, [b]*p* value <0.01, [c]*p* value <0.05 compared to control

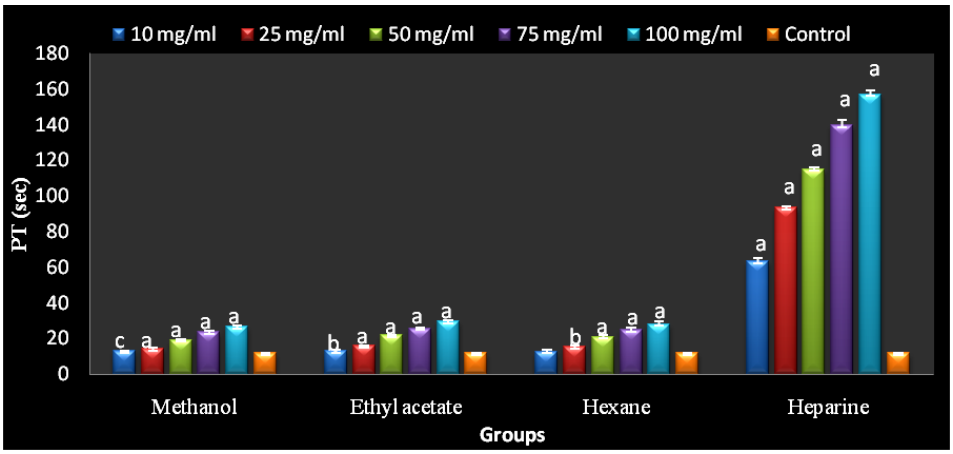

Figure 1: Prothrombin time of normal human plasma treated with *F. Schweinfurthii* extracts:

[a]*p* value <0.001, [b]*p* value <0.01, [c]*p* value <0.05 compared to control

A healthy human's normal value of PT and PTT was 11.8 and 30.20 seconds, respectively. Heparin, as positive control showed considerable anticoagulant activity (PT>60 s, PTT>170 s). The results showed significant PT prolongation (p<0.01, p<0.0001) of the methanol, ethyl acetate, and n-hexane extract at (25, 50, 75, 100 µg/ml) concentrations. In contrast, at a concentration of 10 µg/ml, the methanol, ethyl acetate, and n-hexane extract did not significantly (p>0.05) prolong PT compared with the plasma. In the PTT assay, there was an insignificant prolon-gation (p>0.05) of PTT at a concentration of 10 µg/ml of methanol and ethyl acetate extracts.

Table 2: Results of *in vitro* anticoagulant activity assay (PTT) in a sec of *F. schweinfurthii* extracts.

Sample concentration (µg/ml)	Methanol extract	Ethyl acetate Extract	Hexane extract	Heparine
10	31.29±0.44	31.08±0.67	30.33±0.84	115.66±0.72[a]
25	34.40±0.40[a]	33.06±0.30[b]	32.16±0.4[c]	132.33±1.71[a]
50	37.94±0.95[a]	36.26±0.4[a]	33.33±0.19[a]	147.28±0.80[a]
75	46.23±0.88[a]	45.00±0.23[a]	39.59±1.10[a]	162.65±1.19[a]
100	55.60±0.84[a]	48.61±1.28[a]	43.17±0.68[a]	178.56±1.49[a]
control	30.20±0.44	30.20±0.44	30.20±0.44	30.20±0.44

Note: [a]*p* value <0.001, [b]*p* value <0.01, [c]*p* value <0.05 compared to control

However, n-hexane extract did not significantly prolong PTT at 10 µg/ml and 25 µg/ml concentrations (p>0.05). On the other hand, methanol and ethyl acetate extracts produced significant prolongation (p<0.01, p<0.0001) of PTT at 25, 50, 75, 100 µg/ml concentrations. While, n-hexane extract showed a significant prolongation (p<0.01, p<0.0001) of PTT only at 50, 75, 100 µg/ml.

The highest PT and PTT prolongation times were noted at 100 µg/mL compared with other concentrations under testing. The methanol extract exhibited the highest PTT Prolongation activity compared with other tested extracts. At the same time, ethyl acetate recorded the largest PT prolongation activity.

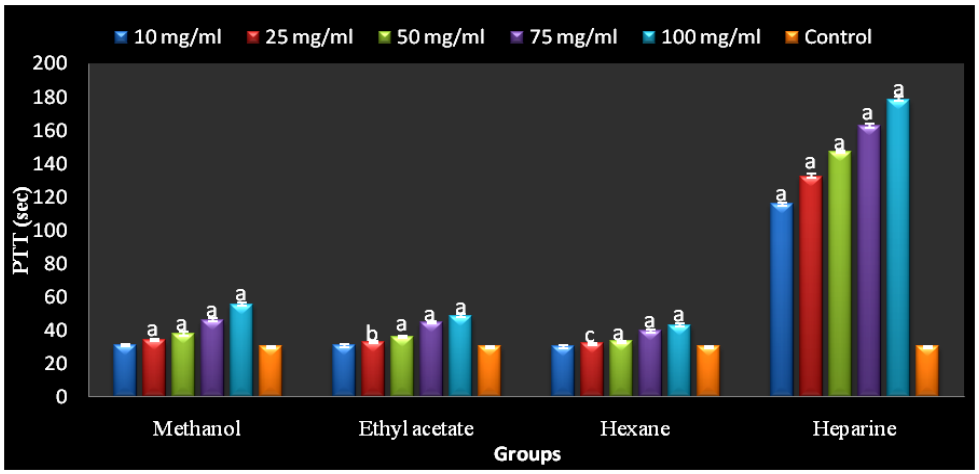

Figure 2: Partial thromboplastin time of normal human plasma treated *F. schweinfurthii* extracts.

[a]*p* value <0.001, [b]*p* value <0.01, [c]*p* value <0.05 compared to control

DISCUSSION

Anticoagulant and procoagulant medications are commonly used in healthy and pathological states, including cardiovascular disease, diabetes mellitus, and bleeding disorders. Although many of these medications have been developed over time, the majority are frequently associated with unfavorable side effects. New procoagulants and anticoagulants are still needed with fewer side effects[13]. The current study considered the *in vitro* anticoagulant effects of *F. schweinfurthii* aerial parts methanol, n-hexane, and ethyl acetate extract using classical PT and PTT assays. The current study examined the *in vitro* anticoagulant effects of *F. schweinfurthii* aerial parts methanol, n-hexane, and ethyl acetate extract using classical PT and PTT assays. The results indicated a considerable prolongation of the PT and PTT by n-hexane, ethyl acetate, and methanol extract depending on the concentration with an optimal prolongation of PT and PTT at 100 µg/mL and the minimal prolongation time was obtained at 10 µg/m. These results agreed with Ismail *et al.*,[14] and Chourasia *et al.*,[15] that *Fagonia cretica* leaves extract and aqueous extract of *Fagonia arabica* and its fractions exhibited a concentration-dependent anticoagulant activity.

A comparison of the effect of the three extracts on the PT and PTT showed that the ethyl acetate and methanol extract exhibited the highest anticoagulant action. These results agreed with Duric *et al.*,[16] that methanol extract showed the highest anticoagulant activity. The largest anticoagulant action was shown with ethyl acetate (medium-polar) and methanol (polar) extracts compared to the non-polar n-hexane extract, suggesting that the primary anticoagulant elements of *F. schweinfurthii* aerial parts are polar. Anticoagulant activity *F. schweinfurthii* aerial parts extracts may be attributed to saponin, flavonoids, tannin, triterpenoids[13,15], and coumarins[17,18]. Coagulation parameters PT and PTT are utilized to identify the clotting mechanism. Prothrombin time (PT) is a useful test for determining how well the components of the extrinsic coagulation pathway are functioning. The PTT is also used to evaluate the activity of components involved in intrinsic and common pathways. In clinical examination, prolonged PTT and PT values imply an abnormality in the activity of particular clotting factors; for instance, an exceptionally prolonged PTT but a normal PT value suggests the need to assay contact pathway factors VIII, IX, and XI.

The common pathway's factors V, X, and prothrombin (factor II) are implicated if both the PT and PTT are affected[19]. Therefore, the extended PT and PTT induced by therapy with *F. schweinfurthii* propose that factors V, X, and prothrombin of the common coagulation pathway activity have been inhibited.

CONCLUSION

According to the study, *F. schweinfurthii* has anticoagulant effects that can be used to treat blood coagulation disorders. Additional *in vivo* study is required to investigate the mechanism by which the anticoagulant components of the plant impact their activity.

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AUTHOR'S CONTRIBUTIONS

Alhaj HA: writing original draft, literature survey. **Raweh SM:** investigation, data interpretation. **Al-Kaf AG:** methodology, conceptualization. **Ibrahim HM:** formal analysis, review. The final manuscript was read and approved by all authors.

DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

None to declare.

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